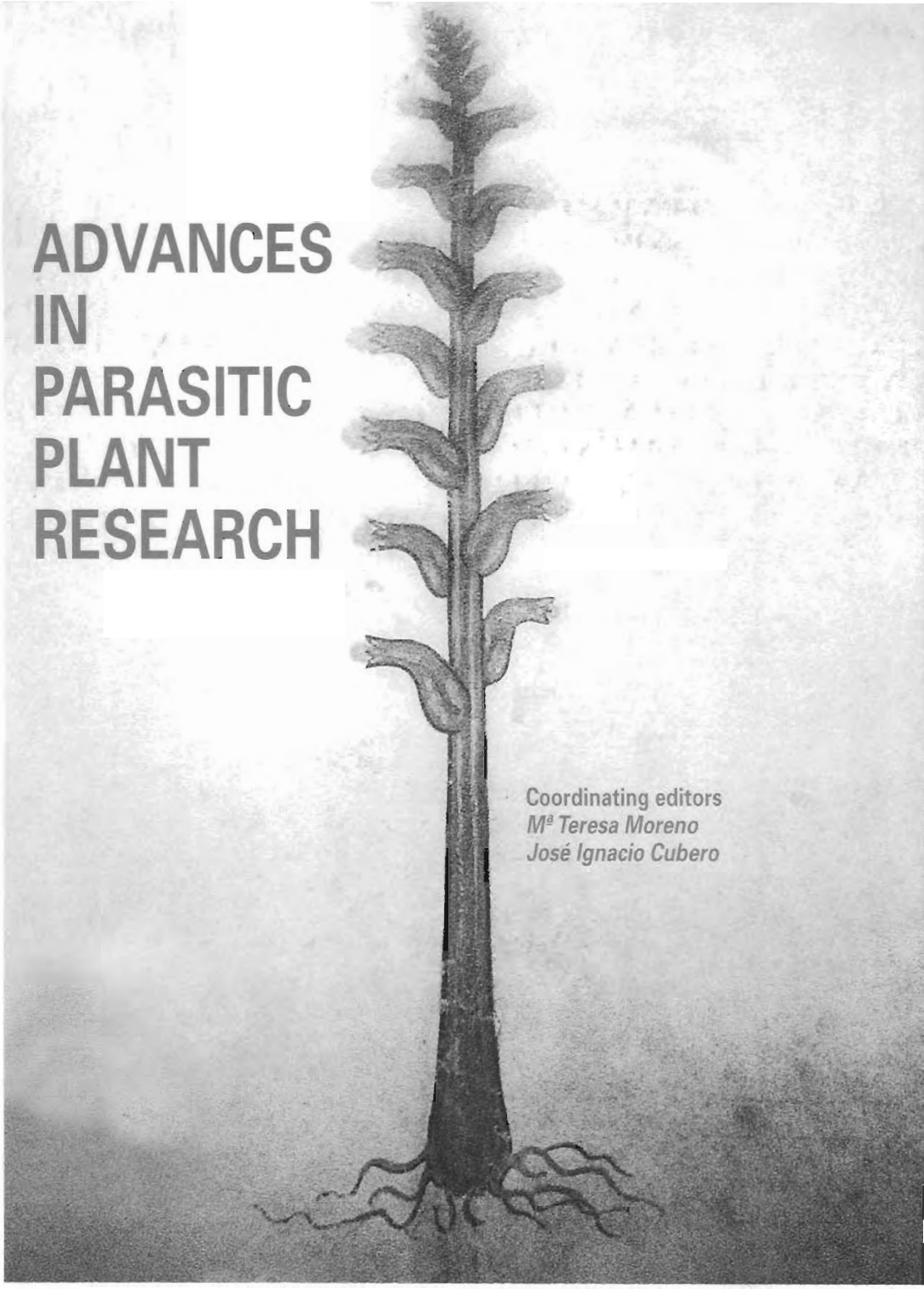
CONGRESOS Y JORNADAS

36/96

ADVANCES IN PARASITIC PLANT RESEARCH



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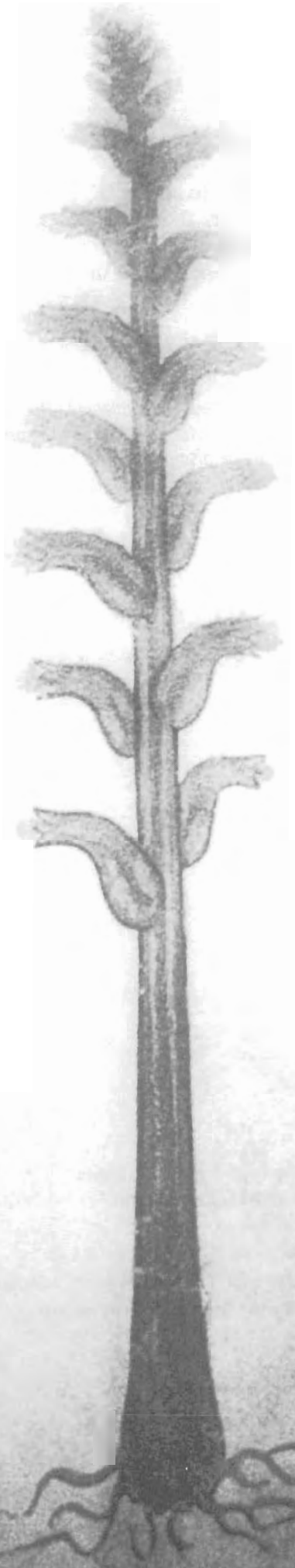
JUNTA DE ANDALUCIA
Consejería de Agricultura y Pesca

Sixth International Parasitic Weed Simposium



Palacio de Congresos y Exposiciones CORDOBA (SPAIN)

6 Sixth Parasitic Widd Symposium

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Advances in Parasitic Plant Research

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Cover: *Orobanche crenata?*, Dioscorides, Vienna, Manuscript, I.A.D.?

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A book is written by authors and published by publishers. But there is also a tedious work usually anonymous: the corrections of drafts and proofs. In a book like the present one, this work particulary heavy because of so many authors and so many disciplines. The coordinating editors are grateful to Mercedes Moreno, Amparo Martínez, Salvador Nadal, Tina Bajo, Ana Torres, Teresa Millán and Fernando Flores for their invaluable help.

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Presentación

Las tierras que los asistentes a este Simposio ven a su alrededor tienen una larga historia de malas hierbas parásitas. Un buen ejemplo es el jopo (Orobanche) que eliminó en el pasado no pocos cultivos e impidió a otros cuantos el establecerse en estas fértiles tierras. Y recientemente, se extiende con mayor rapidez de la que sería deseable por cultivos que, aunque modernos, arraigaron profundamente en la región andaluza. Así pues, nada más claro que el interés que para Andalucía tiene este Sexto Simposio Internacional de Malas Hierbas Parásitas.

La presencia de representantes de nada menos que 29 países pertenecientes a los cinco continentes, indica bien claramente, que no es ni mucho menos un problema local o regional. En realidad, aún cuando el asunto parezca de alta especialización, en pocas ocasiones nos encontramos frente a un problema tan extendido y con una participación tan auténticamente internacional.

Si es cierto lo que se dice de la eficacia de los Congresos, esto es, que lo más importante en ellos es el intercambio personal de opiniones, ideas y datos, no cabe duda que este Simposio de Córdoba ha de ser un éxito. Los cinco Simposios anteriores (que, por cierto, han tenido lugar en cuatro continentes) produjeron no pocos resultados positivos, tanto en el avance del conocimiento básico, como en el de la lucha diaria contra un enemigo sutil pero poderoso. El Sexto los ha de producir por fuerza; no en vano, se han superado todas las previsiones sobre el número de comunicaciones recibidas, lo que ha obligado a una selección para no convertir una reunión científica en algo de imposible realización práctica.

Deseo hacer constar el orgullo que me produce el hecho de que el nombre de Córdoba haya figurado en la lista de prestigiosas ciudades que acogieron técnicos y científicos deseosos de conocer un problema tan viejo, quizás como la propia agricultura.

Paulino Plata Cánovas
Consejero de Agricultura y Pesca

INDEX GENERAL

I.1

PARASITIC PLANT SCIENCE: A QUARTER CENTURY

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Earlier History

The first mention of a parasitic weed in the literature seems to be that of Theophrastus (*circa* 370-285 BC) in his *Enquiry into plants* (8,8,4). It is curious that he gave the name "orobanche" to our actual *Cuscuta*, for the reason given below, broomrape being mentioned as "aimodoron" (ibid., 8.8.5). His descriptions leave little room for doubt. For dodder: "... they are thought to be peculiar to that one, as 'vetch-strangler' [this is the meaning of the greek word "orobanche"] to vetches and bedstraw [*Galium* sp.] to lentils. But the former gains that mastery over the vetches especially because of the weakness of that plant .... it overspreads the whole plant and holds it fast as it were in coils for it is thus that orobanche strangles the plant, and this is the origin of its name". For broomrape: "The plant which springs up straight from the roots of cummin and the plant called "aimodoron" which similarly attaches itself to fenugreek are somewhatmore more peculiar in their habits. "Aimodoron" has a single stem ..... and has on the top a sort of head, while its root is more or less round...".

It is difficult to say when the name "orobanche" was applied to our *Orobanche* for the first time, but Dioscorides already uses it in this way, ".. a stem somewhat red ... hairy, tender, thick, leafless... flower whitish changing to yellowish... its root thick as a finger.... grow among certain legumes, to whom it strangles...". Besides, the wonderful drawings of the Vienna manuscript show without error what is an single stem broomrape. In his comments on Dioscorides, the Spanish physician Laguna (1510?-1583) adds some drawings and also mentions a different greek name, "cynomorion", explaining that "...it is because its root reminds the penis of the dog" (!!) [incidentally, the commonest local name for broomrape in Ethiopia could be translated as "donkey penis"; we are indebted to Chris Parker for this information]. Several botanists of the XVI-XVII centuries as John Gerard for example, recognised the branched and the single stemmed broomrapes, and perhaps some varieties within the latter. Other parasitic weeds, namely *Cuscuta* and *Viscum* are also mentioned and represented in these works.

But all these authors mentioned our parasitic weeds either as botanical curiosities or because of some pharmaceutical properties. Their parasitic nature, although recognised since Theophrastus as indicated above, was not important from the agricultural point of view. Guettard in 1746 recorded by the first time a damage produced by *O. ramosa* on carrots in France; very probably the Spanish agronomist Valcárcel was the first to deal *in extenso* with the subject from a strictly agricultural point of view. Valcarcel described thiem very accurately in 1770 recognizing their parasitic nature and suggesting the only possible control at that time: pulling off the parasitic shoots. The English botanist Sutton observed for the first time in 1798 the connection between the presence of the host roots in the proximity of the *Orobanche* seeds and the germination of the latter, even though this idea was not very clear in his writing. This fact was stated only in the second half of the 19th century after several works (Chatin, 1853; Solms-Laubach, 1868 and Koch, 1887 in Krenner, 1958; Danger, 1887). Cytogenetical works started in the first third of the present century, only consisting in chromosome counts. In many ways the science of parasitic plants started in a modern sense by the classical monography by Kuijt (1968).

It is obvious that the main interest raised until now by the parasitic weeds has been to try to control them when they dare to attack important crops. However, they show many aspects of both theoretical and practical interest such as, among others: their origin and evolution from green plants (i.e. when and how they became holoparasites), their population structures, their evolutionary pathways as crop parasites, their perfect adaptation as obligate parasites, etc. These studies can have a practical side as new ways for their control as parasitic weeds can be developed.

After this short introduction it is licit to ask: is there a *parasitic plant science?* or rather is the knowledge on them is a quilt composed of distinct portions of different disciplines only connected together in International Symposia on Parasitic Weeds?.

matter", although single authors are and will always be present, if only because general reviews will always be required to detect the advances in a certain field.

There is, however, a matter of concern: molecular biology is pervasive in the sense that it produces quick results, avidly needed by Ph. D. students and, in general, people requiring long and/or fast curricula. It is independent, to a certain extent, of the field work, especially hard under adverse or erratic climates. It is fashionable, while classic disciplines as cytogenetics, systematics, histology etc are unfortunately outmoded for most scientific journals. Thus, an increase in the contributions within that particlualr fiels is predictable for the 7th Symposium, if molecular biologists decide to attend the meetings on parasitic weeds. The experience, in this field, is rather negative: when a matter increases in importance, the result is the organization of different meetings, more specialised than the original ones. It happened in Genetics: first International Congresses had a large portion of plant genetics and plant breeding, almost nonexistent in the recent International Conferences in Genetics. Where a map of genes for resistance to *Orobanche* or *Striga* be presented?: in a Parasitic Weed Symposium, where few people will understand it, or in a plant breeding meeting? It would be desirable in both of them, but will there be time and possibilities....?

Thus, it seems necessary to introduce new methods in the study of old problems. This seems to be the best way to reinforce our knowledge in basic disciplines while maintaining or increasing the activity on conventional subjects. The way to produce a well balanced involvement of novel techniques is to project new research work having in mind the use of these techniques, and this is only possible if the teams working on parasitic weed research manage to integrate specialised scientists in their teams, reinforcing the multidisciplinary approach to solve the problem. This is the way all sciences progressed in the past.

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II.

The Origin and the Present: Evolution, Population Structure

II.1

MOLECULAR STUDIES OF PARASITIC PLANTS USING RIBOSOMAL RNA

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ABSTRACT

Molecular evolutionary and phylogenetic studies of parasitic flowering plants have been advanced through the use of nuclear and plastid-encoded ribosomal RNA genes. Phylogenetic analysis of all families of Santalales using nuclear 18S rDNA sequences supports the basal position of Olacaceae, the monophyly of Opiliaceae, the distinctiveness of Loranthaceae and Viscaceae, and the sister relationship between Santalaceae (including Eremolepidaceae) and Viscaceae. These data do not support a close relationship between Santalales and holoparasites in Balanophoraceae, Hydnoraceae, and Rafflesiaceae. High nucleotide substitution rates in these plants hinder their placement in the global angiosperm phylogeny. Some support is obtained for the placement of Hydnoraceae with the paleoherbs (Magnoliidae). Analysis of 18S rDNA sequences for ten genera of Scrophulariaceae and Orobanchaceae indicate the latter family is paraphyletic. The holoparasites *Orobanche, Harveya, Hyobanche* and *Lathraea* appear on a clade with substitution rates higher than related hemiparasites. Plastid-encoded 16S rDNA was PCR amplified and sequenced from representatives of Balanophoraceae, Hydnoraceae, and Rafflesiaceae, thereby providing preliminary evidence for the existence of a plastid genome. The 16S rRNA sequence of *Cytinus ruber* (Rafflesiaceae or Cytinaceae) has mutations at 8.6% of the 1497 sites, yet structural integrity (and likely functionality) is maintained. Formal relative rate tests document that *Cytinus* is more divergent than any of the previously sequenced plant 16S rRNAs. These holoparasitic plants should be viewed as valuable model organisms that can increase our understanding of the mode and tempo of evolutionary change at the molecular level.

Additional key words: Nuclear rDNA, Phylogeny, Evolution.

Molecular Phylogenetic Studies of Santalales

One of the earliest studies to use 18S rRNA sequences in a phylogenetic analysis of angiosperms examined parasitic Santalales (Nickrent and Franchina, 1990). Using direct rRNA sequencing with reverse transcriptase, sequences from representatives of 10 families were analyzed. Although only three parasitic families were examined, the analyses supported the monophyly of Santalales, the sister group relationship between Viscaceae and Santalaceae and the basal position of Olacaceae within the order. This study also showed that 18S rRNA contained sufficient variation to conduct phylogenetic analyses in angiosperms. Since this initial work, 18S sequences of parasites in Santalales have increased rapidly, mainly owing to the advent of the polymerase chain reaction (PCR). Small-subunit rDNA is readily amplified from genomic DNA samples and can then be purified and directly sequenced. Sampling within Santalales has also steadily improved such that currently genomic DNA exists for representatives of all families of the order and, in some cases, all genera within a family.

The following section will discuss the results of preliminary phylogenetic analyses of Santalales using complete 18S rDNA sequences. The order is here defined as the following families: Olacaceae, Loranthaceae, Misodendraceae, Opiliaceae, Santalaceae and Viscaceae. The holoparasite families Balanophoraceae, Rafflesiaceae and Hydnoraceae are often classified in or near Santalales, however, since these relationships are not clear, these families will be treated separately (see below). The methods used to extract DNA, PCR amplify and sequence 18S rDNA, conduct multiple sequence alignments, and generate minimum-length Fitch parsimony trees (via PAUP - Swofford, 1993) are discussed in Nickrent and Soltis (1995). Figure 3 shows the results of parsimony analysis of 18S rDNA sequences from 52 members of Santalales and several outgroup taxa. Specific results are discussed below.

Olacaceae. The family has traditionally been considered the most primitive of the order based upon the presence of two ovular integuments in some taxa and the presence of both autotrophic and hemiparasitic members. Kuijt (1968, 1969) considered the Olacaceae the "plexus" from which all other families in the order were derived. The combination of primitive and specialized features and the very high number of monotypic genera prompted Sleumer (1984) to suggest the family differentiated early during the Cretaceous, prior to the separation of the continents. The Olacaceae is certainly the most problematic one in the order, for indeed extreme variability can be seen in morphological features such as habit (nonparasitic and parasitic), flower sexual condition (unisexual, perfect), petal fusion (distinct, connate), ovary position (hypogynous, epigynous, perigynous), ovular integuments (0, 1, 2), embryo sac type (monosporic, bisporic), and even cotyledon number (2, 3, 4 and 8). Such variation has prompted the erection (and subsequent subsumption) of numerous splinter families such as Aptandraceae, Cathedraceae, Erythropalaceae, Heisteriaceae, Octoknemaceae, Schoepfiaceae, Scorodocarpaceae, Strombosiaceae, and Tetrastylidiaceae. Sleumer (1935 and amended in 1984) divided the family into three subfamilies: Anacolosoideae (formerly Dysolacoideae), Olacoideae, and Schoepfioideae.

At present only seven of the ca. 28 genera in the family are represented in Fig. 3. All genera except *Schoepfia* are basal in the order, which is concordant with previous taxonomic systems. The genera do not form a monophyletic clade, hence the family is paraphyletic. *Schoepfia screberi* Gmelin forms a clade with *Misodendron brachystachyum* DC and this clade appears basal to the Old World Loranthaceae. This same relationship is supported by analyses of the chloroplast gene *rbc*L (Nickrent, 1996) that included five genera of Olacaceae. *Schoepfia* is distinct from other olacaceous genera in possessing aliform-confluent parenchyma (Sleumer, 1984) and by its ratio of tracheid and vessel features (Reed, 1955). Reed (1955) also

noted similarities in the pollen of *Schoepfia* with Santalaceae (a more advanced family of the order). Taken as a whole, it is worth considering possible phylogenetic affinities between *Schoepfia* and the root parasitic Loranthaceae such as *Atkinsonia* and *Nuytsia*. Additional sampling within Olacaceae is required to further address the possible polyphyly of the family. Assistance by colleagues is requested to acquire genera from tropical South America, Africa, and Indomalaya.

Misodendraceae. *Misodendron* consists of ca. 10 mistletoe species parasitic on *Nothofagus* of southern South America. The genus is unusual in having wind-dispersed fruits (via enlarged, plumed staminodes). Relatively little published information on phylogenetic affinities of this monogeneric family exists, although Kuijt (1968, 1969) derives the family from Olacaceae. Results of analyses of 18S rDNA (Fig. 3) and *rbc*L sequences (Nickrent and Soltis, 1995; Nickrent, 1996) show that *Misodendron* clusters with *Schoepfia* (Olacaceae), although connected to it by a long- branch. Given this molecular and biogeographic information, *Misodendron* may represent a relictual taxon that diverged early from loranthaceous or olacaceous stock present on the Gondwanan landmass.

Loranthaceae. Complete 18S rDNA sequences currently exist for 23 of the ca. 75 genera in this family. Results of phylogenetic analyses (Fig. 3) show that the family (with the inclusion of *Schoepfia* and *Misodendron*) is monophyletic An apparent feature of the tree is that significant genetic differentiation has occurred between clades composed of New World mistletoes (e.g. *Gaiadendron, Ligaria, Psittacanthus*, etc.) and Old World mistletoes (*Loranthus, Tapinanthus, Amyema*, etc.). The two mistletoes endemic to New Zealand (*Tupeia* and *Alepis*) show affinity with *Lysiana*, an Australian genus. *Gaiadendron* is classified with *Atkinsonia* in tribe Elytrantheae (Danser, 1933) and shares with it (and *Nuytsia*) N=12, the base chromosome number for the family. These three genera are considered the most primitive in the family (Barlow, 1983). The 18S rDNA data do not strongly support a basal position in the family for *Gaiadendron* (see, however, *rbc*L analyses in Nickrent, 1996). Although we have DNA for the root parasite *Nuytsia*, we have yet to generate any sequences. Tissue for another root-parasitic genus, *Atkinsonia*, has yet to be obtained.

By examining branch lengths, it is apparent that fewer mutations exist among loranthaceous than viscaceous genera. The molecular data strongly support the concept of Barlow (1983) that these two mistletoe families are distinct. Furthermore, more mutations occur among Old World than New World genera of Loranthaceae. A number of relationships support current concepts, such as associations between *Amyema* and *Diplatia*, *Oryctanthus* and *Dendropemon*, etc. Although 18S rDNA does provide some indications of intergeneric affinities, continued sequencing using a faster rate molecule is required to fully resolve relationships in this family. Sequences for *rbc*L for three genera (*Gaiadendron, Moquinella* and *Tupeia*) indicate that this molecule will also provide insufficient phylogenetic signal to address relationships among all genera. Our lab is currently exploring the utility of two chloroplast genes (*ndh*F and *mat*K) as well as 26S rDNA which contains more rapidly evolving domains (expansion segments).

Opiliaceae. Four of the possible nine genera of this family have been sequenced for 18S rDNA: *Agonandra, Cansjera, Champereia*, and *Opilia*. Results of this analysis (Fig. 3) represents the family as a monophyletic clade between the Santalaceae and Loranthaceae. Opiliaceae is one of the most coherent families within the order as reflected by the overall similarity in its wood structure (Reed, 1955). Similarities between Opiliaceae and Santalaceae can be seen in floral morphology (Fagerlind, 1948), embryology (Johri and Bhatnagar, 1960), and haustorial anatomy (Kubat, 1987). Although Hiepko (1979, 1982) published a taxonomic revision of the Old World members of the family, no intergeneric phylogenetic inferences were made, therefore the classification of Sleumer (1935) must be used. Therein, *Agonandra* (with *Gjellerupia*) was placed in its own tribe, Agondandreae. The remaining

seven genera were classified within tribe Opilieae. rDNA analyses place *Opilia*, not *Agonandra*, at a basal position in the family, however, this is supported by only a few steps. In contrast, *rbc*L analyses (Nickrent, 1996) place *Agonandra* at the base of the clade in support of the Sleumer (1935) classification.

Santalaceae and Eremolepidaceae. Pilger (1935) divided the family into three tribes, the Anthoboleae (2 genera - *Anthobolus* and *Exocarpos*), Osyrideae (= Santaleae - 22 genera), and Thesieae (6 genera). Little subsequent taxonomic work has been conducted on higher-level relationships in the family. This analysis utilized sequences from 11 of the 30 genera of Santalaceae. The family does not form a monophyletic group but a grade that eventually culminates in Viscaceae (Fig. 3). Sequences for only two genera (*Osyris* and *Santalum*) were used in the *rbc*L analysis by Nickrent (1996), however, they formed a monophyletic group (with Eremolepidaceae - see below) sister to Viscaceae. Several relationships are worth noting. *Buckleya* and *Pyrularia*, two relictual genera that have representatives in eastern North America and eastern China, form a clade at the base of the family. The morphologically similar north temperate genera *Geocaulon* and *Comandra*, form a clade with *Thesium*. An unresolved polytomy that includes *Osyris*, *Nestronia* and *Santalum* in one clade and *Dufrenoya* and *Dendrotrophe* in another is also present.

Three santalalean genera, *Antidaphne*, *Eubrachion* and *Lepidoceras*, were placed in the family Eremolepidaceae by Kuijt (1988) and allied with primitive Loranthaceae. The results of this 18S rDNA sequence analysis places two representatives (*Antidaphne* and *Eubrachion* - DNA has yet to be obtained from *Lepidoceras)* within the Santalaceae (Fig. 3). *Eubrachion* forms a clade with *Exocarpos*, the one representative of tribe Anthoboleae, and *Antidaphne* occupies a position intermediate between Santalaceae and Viscaceae. Results of analyses of *rbc*L and 18S rDNA sequences (Nickrent and Soltis, 1995; Nickrent, 1996) place both *Antidaphne* and *Eubrachion* on a clade with Santalaceae. These results support the statements made by Wiens and Barlow (1971) that Eremolepidaceae is not closely related to Viscaceae and that the three genera are not related to each other based upon karyological and morphological evidence. Embryological features for these two families, summarized by Bhandari and Vohra (1983), support an association between Eremolepidaceae and Santalaceae. Taken together, these results are intriguing since, prior to the present molecular study, the only aerially parasitic Santalaceae were Old World genera such as *Phacellaria*, *Dendromyza*, and *Cladomyza*. The status of Eremolepidaceae ultimately depends upon the placement of the third genus, *Lepidoceras*, which has resided with viscaceous mistletoes, the Loranthaceae (Kuijt 1968) and later Eremolepidaceae (Kuijt 1988).

Viscaceae. Analyses of nuclear 18S rDNA sequences of the Viscaceae confirms its monophyly, derivation from Santalaceae, and advanced position in the order (Fig. 3). Contrary to Bandahri and Vohra (1983), the family is distinct from Loranthaceae and, relative to it, exhibits increased evolutionary rates. This can be seen by examining branch lengths on Fig. 3, especially for advanced hemiparasites such as *Arceuthobium*. Sequences from both 18S rDNA and *rbc*L have been obtained from several representatives from each of the seven genera in the family. The three species of *Arceuthobium* (Old and New World species) form a clade that is sister to two Australian species of *Notothixos*. *Ginalloa arnottiana* Korth. (from Borneo) clusters with two species of *Korthalsella* (from Hawaii and New Zealand). The next clade is composed of the New World genera *Dendrophthora* and *Phoradendron*. The last clade is composed of two species of *Viscum*. The order of branching for the above major clades is not resolved following bootstrap analysis, hence only the relationships between *Phoradendron* and *Dendrophthora* and *Korthalsella* and *Ginalloa* are strongly supported.

These results differ in several ways from the relationships proposed by Wiens and Barlow

(1971) who used mainly chromosomal and biogeographical information. They proposed two major lines, one composed of *Viscum*, *Notothixos* and *Ginalloa*, the second composed of *Phoradendron*, *Dendrophthora*, *Korthalsella* and *Arceuthobium*. Given their similar distributions and shared karyotype of 12 or 13 large chromosomes, it is logical to derive a relationship between *Ginalloa* and *Notothixos*, as was done by Wiens and Barlow (1971). This relationship is not supported by either rDNA (Fig. 3) or *rbc*L (Nickrent, 1995; Nickrent and Soltis, 1995; Nickrent, 1996) sequence analysis which both link *Korthalsella* with *Ginalloa*. The array of chromosome numbers seen in *Viscum* (N=10, 11, 12, and 13) suggests that extensive genetic variance exists for this feature. The basal position of this genus following bootstrap analysis (results not shown), although not strongly supported, suggests radiation of the major viscaceous lines from a *Viscum*-like ancestor. Further studies of the molecular evolution in this family using 26S rDNA or other molecules is required to elucidate relationships in this family.

Molecular Phylogenetic Studies of Rafflesiales and Balanophoraceae

Over the past century, traditional means of classifying Balanophoraceae, Hydnoraceae, and Rafflesiaceae have met with difficulty owing to the extreme reduction and/or modification of morphological structures that have accompanied the evolution of these lineages. Moreover, the features that remain are often enigmatic, i.e. they are so unusual (derived) as to confound assessment of homology via character state transformation series. The disagreement regarding classification of these families can be seen by comparing three current systems for angiosperms. Cronquist (1981, 1988) placed Balanophoraceae in Santalales and classified Hydnoraceae and Rafflesiaceae together in the sister order Rafflesiales. The system of Takhtajan (1980, 1987) was similar in that Hydnoraceae and Rafflesiaceae were placed in Rafflesiales, however, this order was derived from within subclass Magnoliidae, not Rosidae. Takhtajan (1980) stated that Balanophoraceae (in Balanophorales) were "probably near to and derived from Santalales, but the affinity is not fully clear." The system of Thorne (1992) differed little from the above by classifying Balanophorales near Santalales and Rafflesiales in superorder Rafflesianae.

Resolution of phylogenetic relationships in angiosperms has become increasingly refined using molecular markers such as *rbc*L and 18S rDNA (Chase *et al.*, 1993; Nickrent and Soltis, 1995). For this reason, the analysis of appropriately selected molecular markers holds great promise in placing the holoparasites within the global phylogeny of all angiosperms. Unfortunately, the chloroplast genome of these plants is likely highly modified, hence genes such as *rbc*L are not available for analysis (see below). As already demonstrated for Santalales, 18S rDNA sequences are appropriate molecular markers for addressing phylogenetic relationships. Complete 18S rDNA sequences have been obtained for representatives of Balanophoraceae, Hydnoraceae, and Rafflesiaceae. Analyses of sequences from these three holoparasite families showed increased nucleotide substitution rates as was first observed in 18S rDNA of *Arceuthobium*. Using relative rate tests, it was found that the holoparasites were, on average, 3.5 times faster than nonparasitic and hemiparasitic plants (Nickrent and Starr, 1994). Elevated substitution rates in genes normally considered extremely conservative, such as nuclear ribosomal genes, complicates phylogenetic analysis. For example, when these divergent sequences are included in an analysis with nonparasites, the resulting topologies frequently show aberrations such as "long-branch attractions" (Felsenstein, 1978) that artifactually links clearly unrelated taxa with faster rates. When fewer than 20 nonparasitic "outgroup" taxa are used, the parasites with long-branches migrate to the base of the angiosperm clade, intermediate between the angiosperms and the outgroup Gnetales. This same result is seen using parsimony, neighbor-joining and maximum

Lathraea is the last member to emerge from this large polytomy.

Colwell (1994) conducted phylogenetic analyses of hemiparasitic Scrophulariaceae and holoparasitic Orobanchaceae using nuclear 18S rDNA sequences. Although this molecule usually provides insufficient characters for analysis at the rank of family and below, increased rates of nucleotide substitution in Orobanchaceae provided enough variation to address relationships at this level. Colwell (1994) showed results of analyses of nine ingroup (parasitic) plants, however, subsequent sequencing (Colwell and Nickrent, unpublished) has increased this number to 18. Phylogenetic analysis was conducted using 18 ingroup 18S rDNA sequences (Scrophulariaceae and Orobanchaceae) and three outgroup sequences (*Glycine, Lycopersicon* and *Ipomoea*). Two equally parsimonious minimum-length trees were obtained and the consensus is shown in Fig. 5. A clade containing *Linaria* and *Chamophila* is sister to the remaining Scrophulariaceae plus Orobanchaceae suggesting (as with *rps*2, above) that parasitism arose just once. The topology of this tree is fully concordant with that obtained with *rps*2 and in fact provides further resolution of relationships. The hemiparasites *Pedicularis, Orthocarpus* and *Castilleja* form a clade separate from the holoparasites. Three genera have been placed in either Scrophulariaceae or Orobanchaceae by different taxonomists: *Harveya, Hyobanche* and *Lathraea*. This 18S rDNA analysis shows these three genera to be components of a clade containing three species of *Orobanche*. Although taxon sampling is still incomplete, it appears that members of this clade are undergoing accelerated rates of nucleotide substitution compared with their relatives. The 18S tree also indicates that the genus *Orobanche* is monophyletic, yet it is not closely related to *Epifagus, Conopholis* and *Boschniakia* thus making Orobanchaceae paraphyletic. This study provides evidence that 18S rDNA can be used to address phylogenetic questions in Orobanchaceae. Of the 15 "nontransitional" genera classified in this family, only four have been sampled, hence the inclusion of more Old World representatives is needed to fully address the remaining questions.

General Features of Parasite Plastid Genomes

Among the three distinct genomes in plant cells, the circular plastid genome is certainly the best characterized in terms of structure and function (Sugiura, 1992). Green plants have from one to several hundred chloroplasts per cell and each chloroplast may contain from 7 to greater than 200 plastid genomes (Maguire *et al.*, 1995). The complete chloroplast DNA molecule has been sequenced for *Marchantia* (Ohyama *et al.*, 1986), *Pinus* (Wakasugi *et al.*, 1994), *Oryza* (Hiratsuka *et al.*, 1989), *Nicotiana* (Shinozaki *et al.*, 1986) and *Epifagus* (dePamphilis and Palmer, 1990). The cpDNAs of these plants vary widely in size: 119 kb in pine, 121 kb in liverwort, 134 kb in rice, and 156 kb in tobacco. The genome of *Epifagus virginiana* (L.) Bart. (beechdrops) is only 71 kb owing to extensive losses of photosynthetic genes that have accompanied its loss of photosynthesis (dePamphilis and Palmer, 1990). *Epifagus* has a large single copy region (LSC) of 18 kb (vs. 87 kb in tobacco), a small single copy region (SSC) of 3.6 kb (vs. 18.5 kb) that contains only two genes (Wolfe, *et al.*, 1992), but strangely the inverted repeat regions are present and full sized (25 kb). Of the 42 genes remaining in *Epifagus*, 38 are involved in protein synthesis (e.g. rRNA, tRNA, ribosomal protein genes), yet expression relies upon import of nuclear-encoded tRNAs and RNA polymerase (Morden *et al.*, 1991). The selective maintenance of these specific genes (versus loss of nearly all photosynthetic genes) provided inferential evidence of their functionality, but direct evidence was obtained by Ems *et al.* (1995) using Northern blot analyses. *Conopholis americana* (L.) Wallr. (squawroot), another holoparasite in Orobanchaceae, has also been the subject of molecular studies. Using heterologous probes, Wimpee *et al.* (1991) documented the modification or absence of many photosynthetic genes. Colwell (1994) conducted restriction site mapping of the

plastid genome of squawroot documenting its size as 43 kb, i.e. the smallest ptDNA molecule yet observed in plants. Much of this reduction is due to the loss of one copy of the inverted repeat.

In addition to parasitic Scrophulariaceae and Orobanchaceae, molecular genetic investigations have also been focused upon *Cuscuta*. This genus, sometimes placed in its own family (Cuscutaceae), is widely recognized to share a common ancestor with nonparasitic Convolvulaceae. As shown in Fig. 1, *Cuscuta* (like Scrophulariaceae), includes hemiparasitic species (e.g. *C. reflexa* Roxb.) as well as holoparasitic species (e.g. *C. europaea* L.) that lack thylakoids, chlorophyll, Rubisco and light-dependent CO_2 fixation but (strangely) retain *rbc*L (Machado and Zetsche, 1990). Although the plastid genome of *Cuscuta* is yet to be fully sequenced, significant progress is being made. Bömmer *et al.* (1993). cloned and sequenced a 9 kb portion of ptDNA from *C. reflexa* that includes 16S rDNA, *psb*A, *trn*H, ORF 740, ORF 77, *trn*L, and ORF 55. Later, Haberhausen and Zetsche (1994) cloned and sequenced a 9 kb portion of ptDNA from this same species that included a large portion of inverted repeat A spanning a segment from *trn*A to *trn*H. Although some sequences were identical to *Nicotiana* (e.g. *trn*I), many deletions were observed. For example, *rpl*2 and *rpl*23 were both deleted and ORF2280 was reduced to only 740 bp. These results show that, like *Epifagus*, *Cuscuta* has experienced major deletions in the plastid genome. The complete loss of ribosomal protein genes such as *rpl*2 invites questions about how such the translational apparatus of the plastid functions and its relationship to the other two subcellular genomes.

Plastid rRNA in Parasitic Plants

In plants, the ribosomal cistrons are present on both inverted repeats (when present) and are composed of genes in the following order: 16S, *trn*I, *trn*A, 23S, 4.5S, and 5S (Fig. 2B). This arrangement is extremely conserved, yet some variation can be seen in Orobanchaceae. *Conopholis* retains an intact 16S and 23S rDNA, however, the intervening *trn*I is absent and *trn*A has become a pseudogene (Wimpee *et al.*, 1992). The flanking 4.5S and 5S rDNAs are intact, but the spacers between them are only 70% of the typical length. *Epifagus* also retains intact 16S and 23S rDNA, but both *trn*I and *trn*A have become pseudogenes while conserving the length of the entire cistron (Wolfe *et al.*, 1992).

Prior to 1995, there were no published data available regarding the presence of a plastid genome (or genes) in Balanophoraceae, Hydnoraceae or Rafflesiaceae. Despite experiencing extensive losses in photosynthetic and other genes, both *Epifagus* and *Conopholis* have intact and functional ribosomal cistrons. Given this, it was reasoned that if any genes were present in these holoparasites, very likely they would be rDNA. The goal of these initial studies was to use PCR to amplify 16S rDNA from each of these lineages.

A multiple sequence alignment was conducted on 16S rDNA using cyanobacterial outgroups as well as published plant sequences. Conserved regions were identified and oligonucleotide primers developed for PCR and sequencing. PCR was conducted using the 8 forward [GGA GAG TTC GAT CCT GGC TCA G] and the 1461 reverse [GGT GAT CCA GCC GCA CCT TCC AG] primers (numbers based upon position on tobacco). To date, 16S rDNA has been amplified from genomic DNA samples of representatives of Balanophoraceae, Hydnoraceae and Rafflesiaceae (Nickrent *et al.*, 1995). We have also amplified 23S rDNA and the spacer region between the 16S and 23S rDNA in several taxa indicating that a large portion (if not a full) ribosomal cistron is present. Although compelling, these data do not yet prove the existence of a plastid genome since the possibility of migration to nucleus has not been excluded.

A secondary structure of the 16S rRNA of *Cytinus ruber* (Rafflesiaceae or Cytinaceae) is presented in Fig. 6 based upon the model of Gutell *et al.* (1985) for *Zea*. This model corresponds well to previously

reference) must be an unambiguous outgroup to taxa 1 and 2 for this test to be meaningful. For this reason, *Pinus* was chosen as the reference since the topology shown in Fig. 7 does not place *Cytinus* with the dicots.

The results of the relative rate tests shown in Fig. 8 clearly indicate the gross rate asymmetry found in the branch leading to *Cytinus*. In the study by Wolfe *et al.* (1992), most substitutions were seen along the *Epifagus* and *Conopholis* branch, hence the authors concluded that the rate of substitution was higher in these holoparasites. The formal relative rate tests reported here do not show these two plants to exhibit any greater rate asymmetry than in comparisons of other plants (such as *Vicia*).

Conclusions

This paper has focused upon the utility of nuclear and plastid ribosomal RNA in molecular phylogenetic and molecular evolutionary studies of parasitic angiosperms. The use of nuclear 18S rDNA in examining the phylogeny of the component families and genera of Santalales has been demonstrated. This molecular phylogeny clearly indicates the progressive evolution from root parasitic (and nonparasitic) Olacaceae to the most advanced clade, the aerially parasitic Viscaceae. Mistletoes are represented by at least four, independently evolved groups (Viscaceae, Loranthaceae, Misodendraceae, and Santalaceae/Eremolepidaceae). Data from nuclear-encoded rDNA and plastid-encoded *rbc*L indicate that *Antidaphne* and *Eubrachion* (Eremolepidaceae) are best classified within Santalaceae. The difficulty in placing the holoparasite families Balanophoraceae, Hydnoraceae, and Rafflesiaceae within the overall classification of angiosperms can be attributed to their extremely derived vegetative and floral features combined with very high rates of molecular evolution. Preliminary evidence from 18S rDNA analyses place Hydnoraceae with the paleoherbs (Magnoliidae).

Nuclear 18S rDNA sequence analysis is in agreement with recent classifications that recognize Orobanchaceae as a component of Scrophulariaceae. 18S rDNA data are concordant with analyses of *rps*2 in that both recover a monophyletic group composed of rhinanthoid Scrophulariaceae and Orobanchaceae, thus indicating the unique origin of parasitism in the family. Genera traditionally considered transitional between Scrophulariaceae and Orobanchaceae (*Lathraea, Harveya* and *Hyobanche*) emerged as components of the *Orobanche* clade. The utility of 18S rDNA sequences in addressing subfamilial phylogenetic relationships has been demonstrated but not fully realized owing to incomplete sampling.

Holoparasitic plants (such as *Epifagus* and *Conopholis*) have undergone extreme reorganization of the plastid genome, mainly manifested by the loss of photosynthetic genes. Sequences from plastid rDNA from representatives of Balanophoraceae, Hydnoraceae, and Rafflesiaceae have been obtained thereby suggesting that these plants have also retained a plastid genome. These plants have the most divergent 16S rRNAs ever documented, yet structural studies provide evidence that these molecules are still functional. Extreme rate heterogeneity, as compared with other vascular plants, was demonstrated for 16S rDNA in *Cytinus ruber* using relative rate tests. This amount of change at highly conserved ribosomal loci provides unprecedented opportunities to study the molecular evolution of the plastid and nuclear genomes. For example, the study of these "fast rate" rRNAs can provide general insight into the structure and function of all rRNA molecules by providing compensatory mutations in regions previously thought to be invariant. Unlike *Orobanche* or *Striga*, which are the topic of many papers at this symposium, many of the plants discussed here are rare and endangered. For this reason, they are worthy of conservation efforts and should be viewed as valuable model organisms that can increase our understanding of the mode and tempo of evolutionary change at the molecular level.

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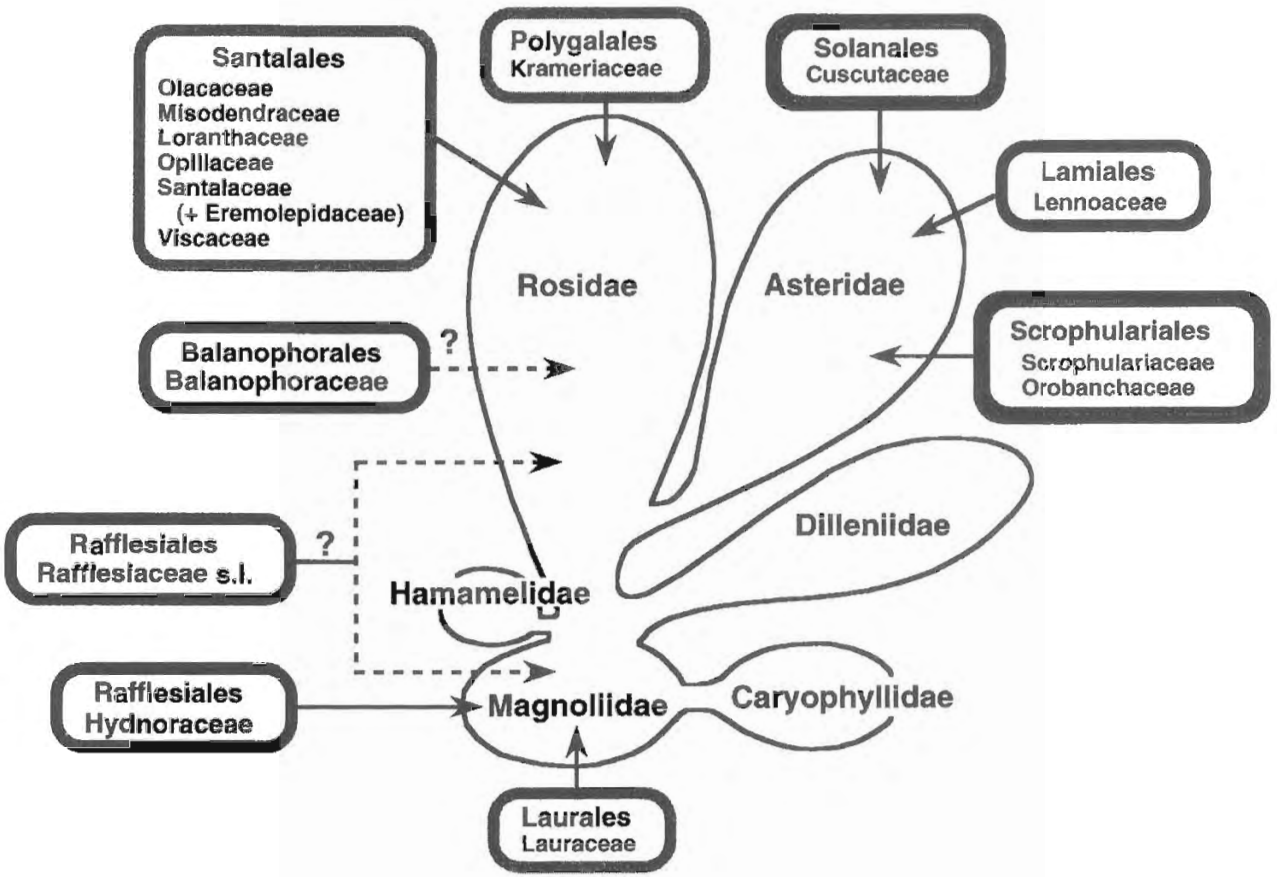

Figure 1

Occurrence of haustorial parasitism in angiosperms mapped upon the balloon phylogeny of subclasses proposed by Cronquist (1981, 1988). The presence of hemiparasitism is indicated by grey borders surrounding Santalales and Polygalales and holoparasitism by means of black borders. Both trophic conditions occur in Cuscutaceae and Scrophulariales. Concepts derived from results of molecular analyses (this paper) are incorporated, such as affinity of Hydnoraceae with Magnoliidae and inclusion of Eremolepidaceae in Santalaceae.

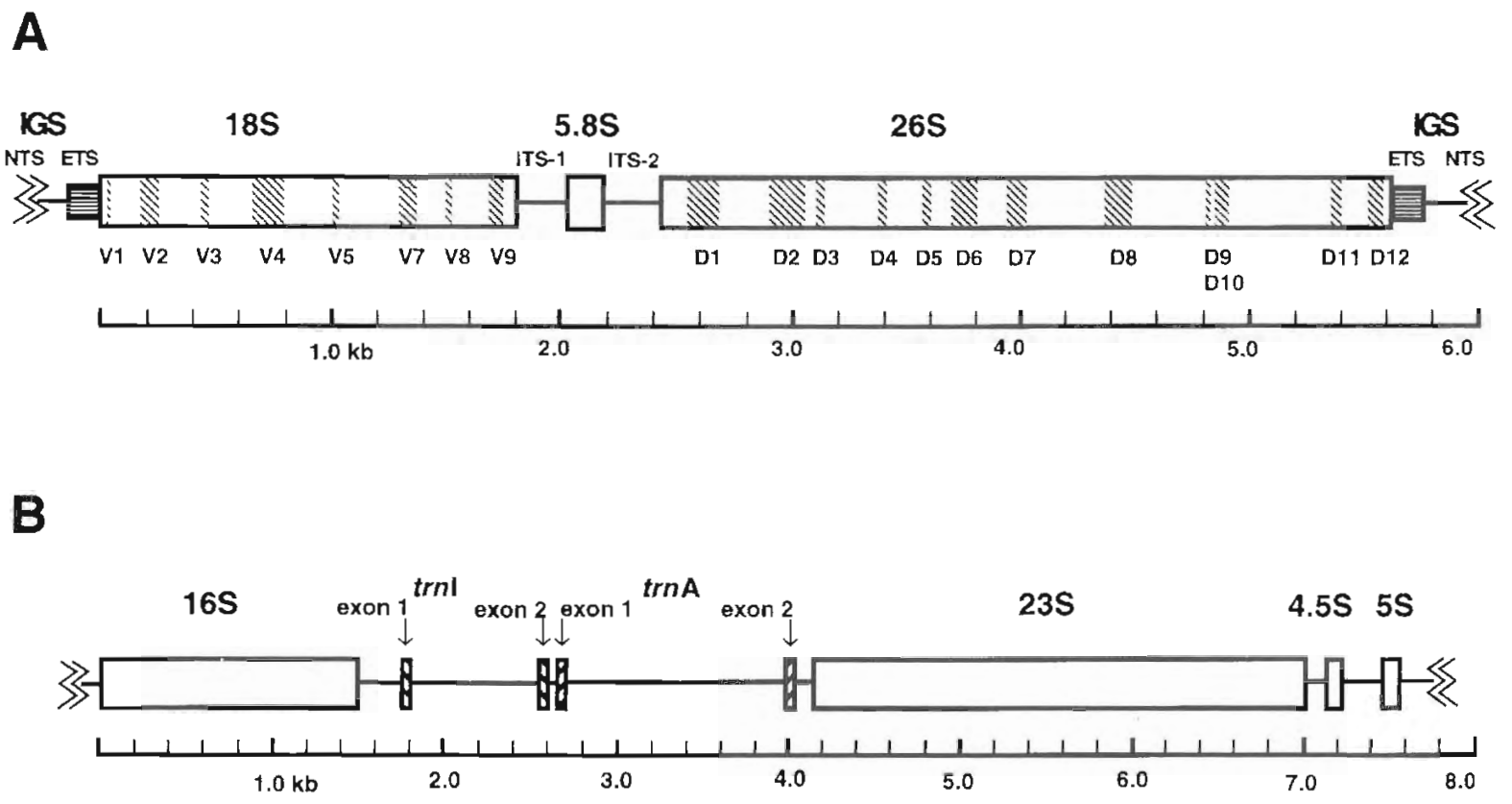

Figure 2

Comparison of plant nuclear and plastid ribosomal DNA cistrons. A. Nuclear rDNA cistron showing small-subunit (18S), large-subunit (26S), and 5 8S rDNAs. Also shown are the intergenic spacer (IGS), external transcribed spacer (ETS), nontranscribed spacer (NTS) and the internal transcribed spacers (ITS-1, -2). Variable domains are shaded (V1-V9 on 18S, D1-D12 on 26S). B. Plastid rDNA cistron as found in tobacco and most other higher plants. The spacer between the small-subunit (16S) and large-subunit (23S) rDNAs contains two tRNA genes ($trnI_{GAU}$ and $trnA_{UGC}$), each containing introns.

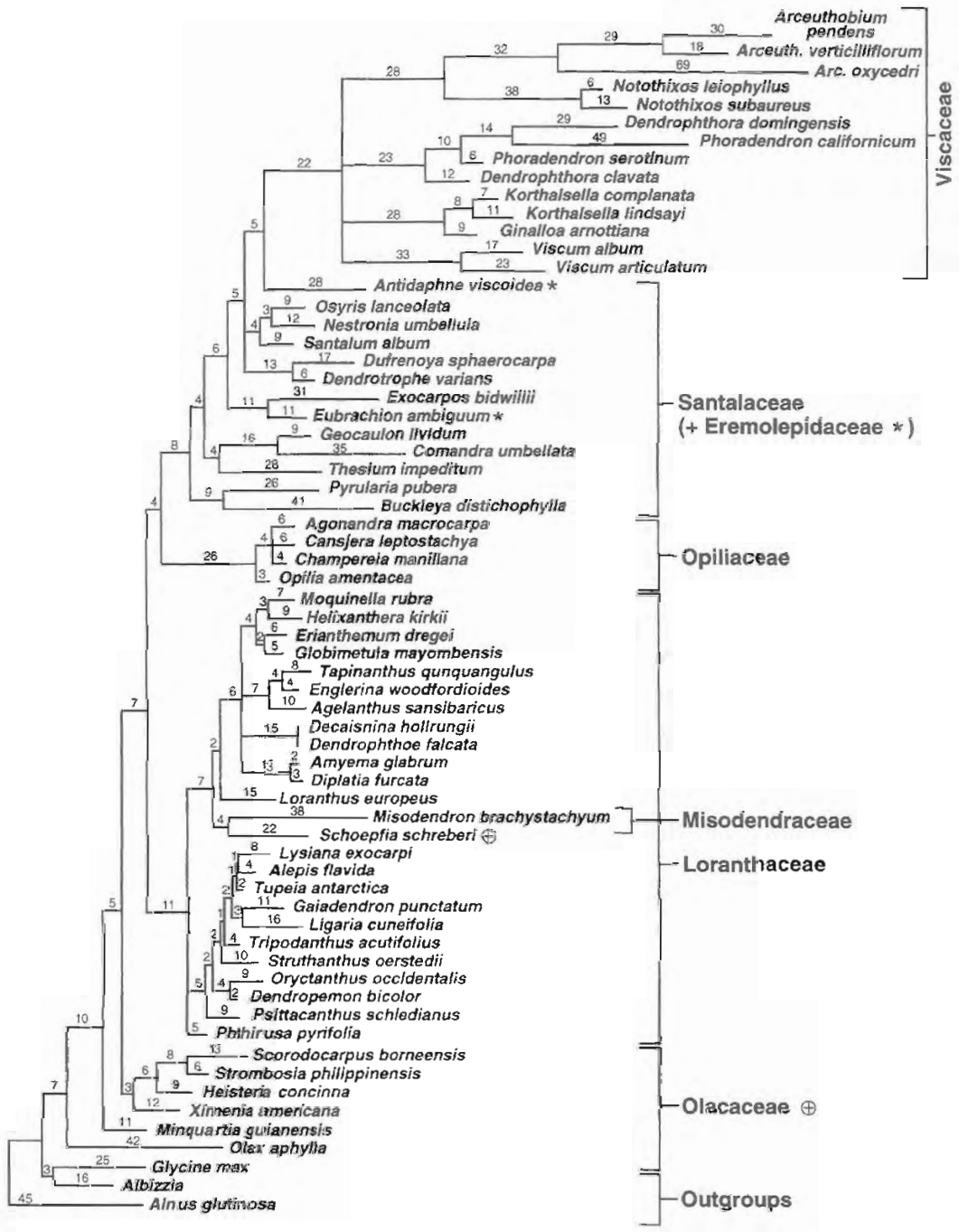

Figure 3

The strict consensus tree derived from a heuristic search with 62 18S rDNA sequences from representatives of Santalales. A total of 144 trees of length 1538 steps were found and are summarized by this consensus phylogram where branch lengths are indicated. The consistency index (minus uniformative sites) is 0.331 and the retention index is 0.610. All Olacaceae are found at the base of the tree with the exception of *Schoepfia* ( ) which is sister to Misodendraceae within the Loranthaceae. Eremolepidaceae, represented by *Antidaphne* and *Eubrachion* (*) are components of Santalaceae (see text).

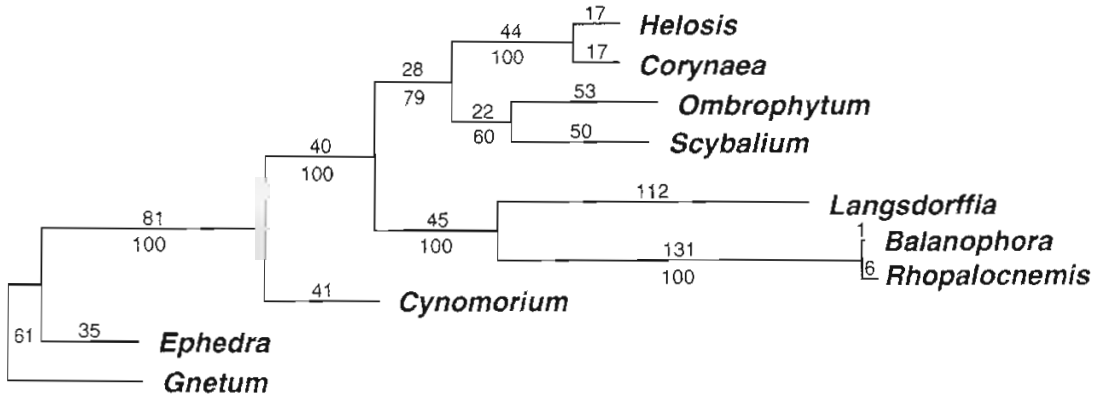

Figure 4

The single tree of length 784 steps found via a branch and bound search of 18S rDNA sequences of Balanophoraceae (and *Cynomorium*). The consistency index (minus uninformative sites) is 0.685 and the retention index is 0.677. Number of steps are indicated above and bootstrap values (from 100 replications) are indicated below the branches.

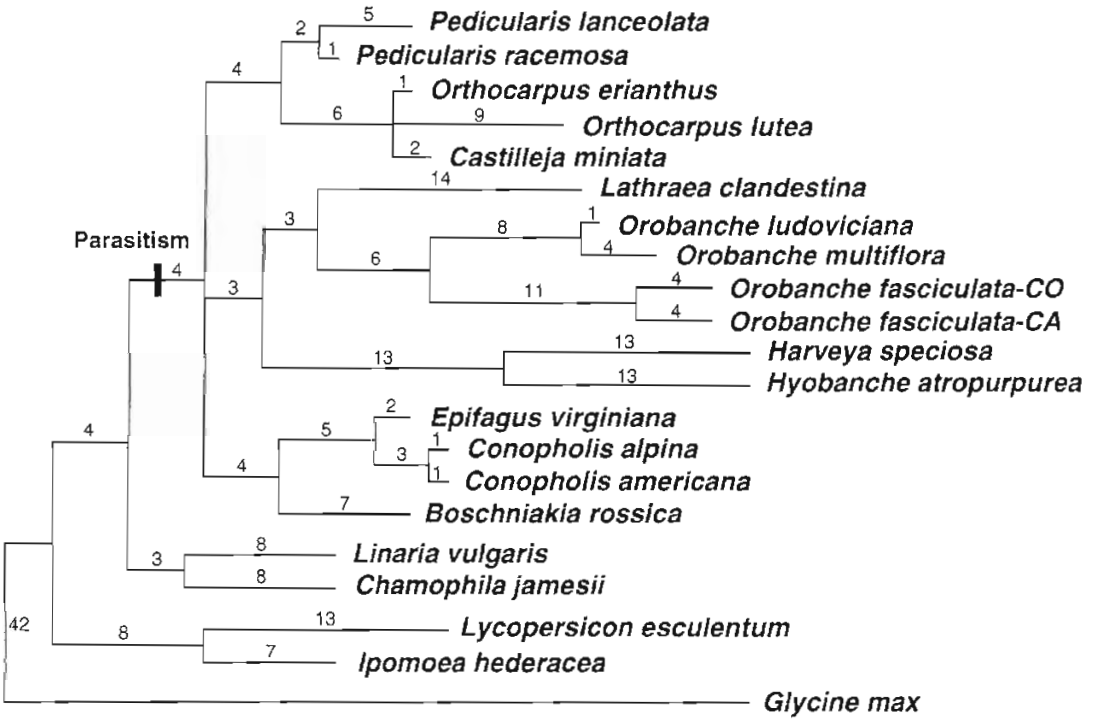

Figure 5

The strict consensus 18S rDNA tree derived from a branch and bound search with 18 sequences from representatives of Orobanchaceae and Scrophulariaceae. Four trees of length 246 steps were found and are summarized by this consensus phylogram. Number of steps are indicated above the branches. The consistency index (minus uninformative sites) is 0.553 and the retention index is 0.643. The evolution of parasitism marks a monophyletic group composed of rhinanthoid Scrophulariaceae and Orobanchaceae.

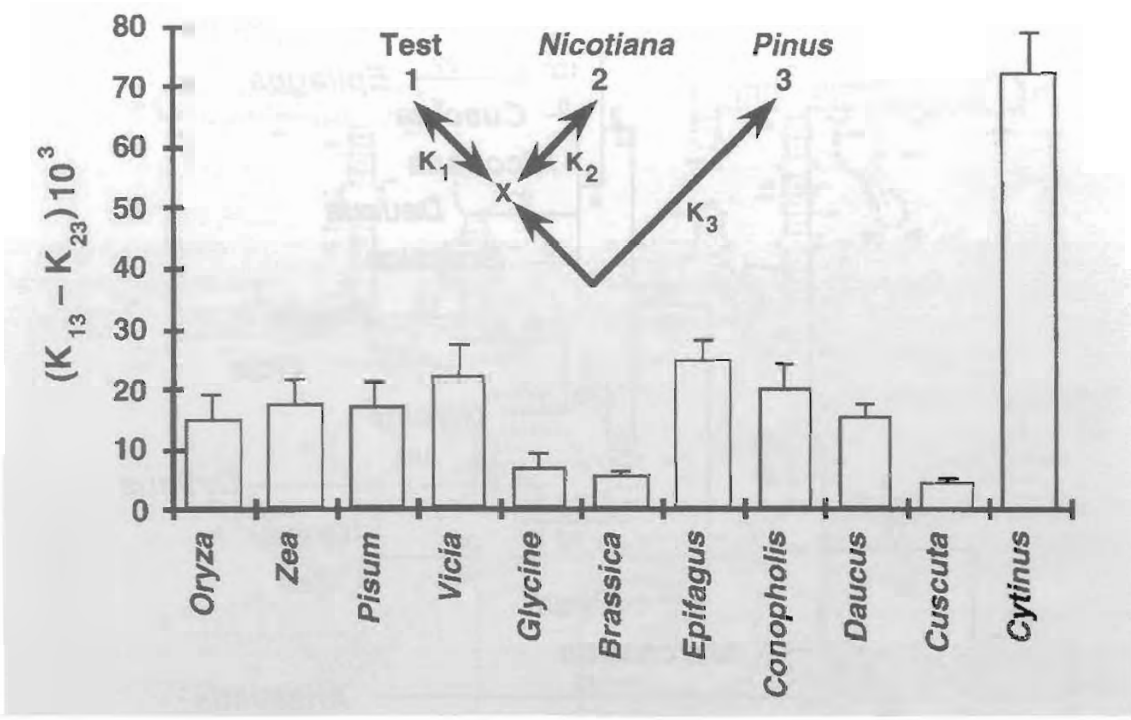

Figure 8

Histogram showing results of relative rate tests using plastid 16S rDNA sequences from 12 angiosperms and *Pinus* (as the reference taxon 3). The three-taxon tree uses K_1, the number of nucleotide substitutions per site for taxon 1, K_2 for taxon 2 and K_3 for taxon 3. The difference in nucleotide substitutions per site ($K_{13} - K_{23}$) is multiplied by 1000 for graphical purposes. Standard error values are included above the bar for each taxon.

II.2

EVOLUTION AND TAXONOMY OF AGRONOMICALLY IMPORTANT *Striga* SPECIES

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ABSTRACT

Taxonomic studies of the taxa clustered about *Striga asiatica*, *S. hermonthica*, and *S. gesnerioides* are presented based on a cladistic study of African plants. The *S. asiatica* cluster includes *S. elegans*, *S. hirsuta*, and *S. lutea*. *Striga asiatica* is considered to be derived from a native species near *S. elegans*. Artificial hybridization indicates a close relationship between *S. hermonthica* and *S. aspera*. Several host morphotypes of *S. gesnerioides* were examined. While each morphotype exhibits features of host specificity, branching, and corolla color, there is little basis for any formal taxonomic recognition.

genera *Lathraea*, *Harveya*, and *Hyobanche* have an intermediate position between the Rhinanthoideae and the Orobanchaceae. He (Minkin, 1987) concluded that the three genera cannot be clearly assigned to either family. Minkin's results also showed that *Buchnera* and *Striga* are closely related taxa, confirming Musselman's (1987) hypothesis. Current research by dePamphilis (personal communication) and Wolfe (personal communication) is certain to help clarify relationships within the witchweeds.

Origin and spread of weedy *Striga*

A theory of the probable center of origin and spread of a weedy *Striga* was first proposed by Rao and Musselman (1987). They suggested that with the introduction of agriculture and domestication of native grasses as cereal crops, the cereal-*Striga* species associated with these grasses became domesticated. Put another way, the crop species evolved from native grasses and the witchweeds evolved from native *Striga* species. Sorghum is the crop with which *Striga* is probably associated in its evolutionary history. At least *S. hermonthica* appeared to have originated in the same area where sorghum originated, the Sudano-Ethiopian region (Musselman, 1980; Harlan and Stanler, 1976; Mulatu and Kebede, 1991), and moved along the routes of introduction of its host from the Sudano-Ethiopian region to different parts of Africa and Arabia. This hypothesis is based on the fact that it is in sorghum among the various hosts of witchweeds that resistance mechanisms are best known. The crops associated evolutionarily with weedy *S. gesnerioides* and *S. asiatica* are not known but it is likely to be a grain in the case of *S. asiatica*, and cowpea (*Vigna unguiculata*) with *S. gesnerioides*.

Species of *Striga* prefer sunny, naturally disturbed grassland habitat in the semi-arid regions. Under these conditions ruderal species like *S. hirsuta*, *S. brachycalyx*, and *S. aspera* flourish and are found in high frequencies especially on the edges of small pools (depressions). In addition to preference for agricultural habitats, the breeding patterns of most witchweeds favor the establishment of large populations from only a few founder plants. Most *Striga* species are autogamous. Autogamy has been well documented for *S. asiatica* and *S. gesnerioides* (Musselman et al., 1982). In these species, the pollen grains form a massive sticky layer that covers the stigma and possibly eliminates the chances of cross pollination. Transfer of pollen probably takes place even before the flowers open. Inbreeding in these species results in the development of host specific strains as is shown for *S. gesnerioides*, or the development of subspecies as evident in *S. bilabiata*.

The second breeding strategy (allogamy) is found in *S. hermonthica* (Safa et al., 1984) and *S. aspera* (Musselman et al., 1991). These two species might share common insect pollinators at least in parts of Africa. A third type of breeding system in *S. forbesii* involving distyly has been reported by Knepper (1989) and Knepper et al. (1991) and deserves further study.

Within the genus there is geographical and host-induced variation (Bharathalakshmi et al., 1990). The two types of variation can be expressed within (intraspecific) or among (interspecific) species. Geographical variation is the difference among species, host-specific strains, or demes of the same taxon parasitizing the same host but growing at different geographical locations. On the other hand, the host-induced variation is the difference among species, host-specific strains, or demes of the same species gathered from a single locality or at the same latitude but parasitizing different hosts. There is a third type of variation, that established by the species since its divergence from its ancestor and during its evolutionary history. This is the species induced variation which is a result of the combined effect of shifting to a new host and a new habitat. It represents the statistically significant difference in the measured variables between two or more species under similar conditions, i.e., parasitizing the same host and growing at the same locality.

This paper is part of a larger study on the evolution and systematics of *Striga* in Africa. We have emphasized three groups of witchweeds clustered around *S. hermonthica, S. asiatica,* and *S. gesnerioides*. In addition, we present preliminary data on possible evolution from indigenous to weedy species.

Materials and Methods

Cladistic analysis was conducted by Mohamed (1994) as part of a study of African witchweeds. Cladistic analysis is presented in Figure 1. *Buchnera, Cycnium, Rhamphicarpa, Melasma*, and *Thunbergianthus* from the Buchnereae and *Scoparia* from the Digitaleae were used as outgroups. The latter taxon was included to provide a non-parasitic, distantly related taxon. *Buchnera* was chosen because it is presumably closely related to *Striga* (Musselman, 1987; Minkin, 1987) and probably its sister group. The others were used to provide features necessary to polarize characters within the group under study.

Six vegetative and seven floral (reproductive) characters were quantified for statistical analysis. These were A=Stem height (cm); B=Number of branches; C=Leaf length (mm); D=Leaf width (mm); E=Internode length (mm); F=Inflorescence length (cm); G=Number of flowers open/inflorescence; H=Lower bract length (mm); L=Calyx tube length (mm); M=Calyx teeth length (mm); N=Corolla tube length (mm); O=Lower corolla lobe length (mm); and P=Upper corolla lobe length (mm). Weighting some of the characters, i.e., requirement of a germination stimulant, host specificity, succulence, haustorium size, and plant color, did not change the tree topography when all characters were assigned equal weight. Even taxa like *S. angustifolia* that are uniquely defined by a lack of need for a germination stimulant and *S. gesnerioides* and *S. lepidagathidis* that are the only species with succulent stems, dicot hosts, and reduced chlorophyll, retained the same position on the cladogram with or without weight.

This analysis was based on herbarium specimens collected in Botswana, Burkina Faso, Cameroon, Mali, Nigeria, South Africa, and Sudan between 1987-89, and specimens cited in Mohamed (1994).

The procedure of multivariate statistics (multiple analysis of variance) was used to compare the effect of the groups (species, geographical locations, hosts) on the measured variables and to determine the correlated responses of the dependent variables. The analysis requires and must be performed on normally distributed data; hence, transformations were made whenever necessary. Since thirteen measured variables were to be assessed, the biological alpha level was adjusted to 0.004 (0.05/13).

Upon the completion of the analysis, a matrix was designed to enable comparison of any two groups (Table 1). Results were plotted on the matrix and two of its axes were similarly labeled with the species, subspecies, host specific-strain, or the geographical locality in question. Latitudes were plotted in ascending or descending order to detect and associate the overall changes (if any) in the number of measured variables with latitudes. However, no attempt was made to trace and correlate the changes in individual variables with changes in latitude which was not the subject of this study. In these matrices only the significant variables are shown.

Hybridization studies were conducted under complete insect exclusion in a screenhouse at the International Institute of Tropical Agriculture, Ibadan, Nigeria. Staggered planting times of maize cultivars 8333-1 and 8322-13, in *S. aspera* and *S. hermonthica* infested soil in pots were made to obtain parasite flowers ready for crossing at the same time. At flowering, emasculation was done by the removal of the corolla tube and affixed stamens.

Pollen was obtained from the male parent by cutting open the corolla tube and collecting the mass of whitish pollen grains on an inoculating needle. Pollen was immediately transferred to the

be maintained under sympatry, and because *S. hermonthica* is an outbreeder, the two strains should express differences that reduce the chances of interbreeding, in other words, character displacement. The differences between the sorghum and the millet strains were mainly floral. On the average, the two populations differed significantly in 3.67 variables per locality. This could reduce the chances of cross pollination under sympatry, and if established, could lead to genetic isolation. Although host-specificity reduces the host range of the parasite it may lead to successful attachment and better adaptability to a given host under specific conditions. If we consider a host like sorghum that developed resistance to the parasite, host-specificity and virulence in the parasite are then of great selective advantage. In areas where sorghum and millet were grown in the same or adjacent fields, host-specificity broke down (Ramaiah, 1984; Rao and Musselman, 1987), showing that different forms of *S. hermonthica* have not become fixed due to cross pollination and exchange of genes between the two populations (Ramaiah, 1984).

The possible effects of host, geographical location, and their combined effects of (host/geographical location) were compared using herbarium specimens of *S. hermonthica* gathered at three latitudes and from two hosts (sorghum and millet). On average the two populations differed significantly in 3.67 variables per locality. Possible geographical variation at the host-specific strain level (sorghum and millet strains) were obtained when *S. hermonthica* plants collected from the same host were compared at different geographical localities. These populations exhibited an average difference of two variables per site, meaning that *S. hermonthica*, regardless of its host, is fairly homogeneous over its range.

Effects of interaction between host and geographical location on variation were obtained when *S. hermonthica* plants parasitizing sorghum and millet were compared at different geographical localities. Here the number of significant variables averaged three per site, slightly less than that attributed to host induced variation at a given locality. It follows that the intraspecific variability in *S. hermonthica* was higher than the interspecific variability (variation among host-specific strains is higher under sympatry than under allopatry). This is consistent with the findings of Musselman et al. (1991) who studied differences among populations of *S. hermonthica* and concluded that they exhibit considerable intrapopulational variation in contrast to *S. asiatica* and *S. gesnerioides* (both inbreeders) which are relatively uniform within a given population.

Overall, *S. hermonthica* showed a high number of significant variables but for any two sites or host-specific strains the number of significant variables was relatively low suggesting that it was homogeneous over its range as is expected for an outbreeder.

It seems that most of the variability within *S. hermonthica*, although low, was caused by geographical effects rather than host-specificity. Bharathalakshmi et al. (1990) attributed most of the genetic variability in *S. hermonthica* to geographical separation rather than to host specialization. Even what could be interpreted as host effect was, in fact, a variation induced by climate before the parasite was successful in invading the host, i.e., the variation was established first, then the host was attacked.

Striga aspera

Herbarium specimens of *Striga aspera* (Willd.) Benth. parasitizing various native grasses from seven localities between latitude 07 degrees 10' and 13 degrees 55' N were examined to show the possible geographical effect on variation. In general, 10 variables exhibited significant differences ($P < 0.0012$), 60% of which were vegetative. Also, when any two sites were compared, the number of significantly different vegetative variables was more than double the floral ones, so that variation in *S. aspera* was primarily vegetative. There was evidence

of an increase in the number of significant variables with changes in latitude.

Like *S. hermonthica*, *S. aspera* is an outbreeder (Musselman et al., 1991). Among populations gene-flow is expected to maintain a certain level of homogeneity across the species range. This is consistent with the results obtained.

A total of 385 and 315 measures for each variable were taken from populations of *S. hermonthica* and *S. aspera* respectively, and analyzed to estimate their overall differences. The plants were gathered from different hosts and fairly represent the geographical range of the two species (between latitude 3 degrees 00' and 14 degrees 00' N). Of the thirteen variables, ten were significantly different (P=0.0001), with no significant differences in the number of branches, inflorescence length, and the calyx teeth length.

It is difficult to assess the magnitude of the variation due to hosts in *S. hermonthica* and *S. aspera*, because the former commonly parasitizes cereal crops while the latter parasitizes wild grasses, i.e., the two species do not occur on a single host at the same site. In the few instances where these data were obtained, it was insufficient for a multivariate analysis and the results might be misleading. However, in a preliminary analysis the host was found to have some effects. When the two species were compared on similar hosts they showed less differences than when compared on different hosts.

Kenfack et al. (1996) have shown that in northern Cameroon *S. aspera* has the broadest host range of any species in the genus. If this broad host range is a primitive condition, one could predict that a derivative of *S. aspera* adapted as a weed would have a narrower host range. This seems to be the situation in *S. hermonthica*. Further host range studies in *S. aspera* are needed.

Striga hermonthica and *S. aspera* were similarly sampled from herbarium specimens collected at four geographical localities and then the data was assessed to quantify the variation between the two species under similar environmental conditions. Differences already established between the two species (species induced variation) were similar to those reported with only slight changes in the means of the ten significant variables.

Geographical variation in *S. hermonthica* and *S. aspera* were expressed in eight significant variables (P <0.0025), five of which were vegetative.

This study provides evidence that the differences already established between *S. hermonthica* and *S. aspera* are quantitative and greater than those attributed to host and geographical location.

Herbarium specimens of *Striga hermonthica* and *S. aspera* gathered at four latitudes were analyzed to quantify the possible effects of the above three factors on variation. Results summarized: 1) Variation attributed to species effect under similar geographical conditions, i.e., when *S. hermonthica* and *S. aspera* were compared at the same latitude. The magnitude of this variation averaged 7.5 significant variables at each site. 2) Variation attributed to geographical separation at the species level were obtained when populations within the same species were compared at different geographical locations. This variation averaged 3 and 3.83 variables per site for *S. hermonthica* and *S. aspera* respectively, intimating that they are both homogeneous over their range. 3) The combined effects of latitude and species induced variation were obtained when *S. hermonthica* and *S. aspera* were compared at different latitudes. The average variation here was 7.75 significant variables per site, which was only slightly higher than that attributed to species effect at the same latitude. It seems that the two species expressed the same magnitude of variation when they occur sympatrically and allopatrically (7.5 and 7.75 significant variables respectively). They are outbreeders and among their significantly different variables were those associated with the corolla tube length (N), the lower (O), and upper (P) corolla lobes. Under sympatry, the corolla tube length is significantly different among them in

three out of four sites. The lower corolla lobe length is significant in all four cases while the upper lobe is significant in only one case. Differences associated with the corolla dimensions probably reduces the chances of interbreeding under sympatry. Parker (1991) described from East Africa specimens of *S. hermonthica* with distinct corolla tube morphology where the bend is about half way up the tube.

In cases where there was no significant difference in corolla dimensions the two species might interbreed; In fact the work of Musselman et al. (unpublished) produced the first hybrid in the genus *Striga* between *S. hermonthica* and *S. aspera* with viable seeds. They also noted where the species occur sympatrically some plants seem to have intermediate characters between the two species and can be identified as either of them. Natural hybridization between them seems almost certain since *S. aspera* is visited by butterflies, moths and bees similar to *S. hermonthica* visitors reported in Musselman et al. (1983).

Accumulating data from our crossing experiments in West Africa (Table 2), clearly indicate that these two taxa are interfertile. These results could be interpreted to support the hypothesis that *S. hermonthica*, known only from agroecosystems, might be derived from *S. aspera*. Molecular systematic studies are planned to provide additional data on the relationship between *S. hermonthica* and *S. aspera* although the preliminary data from DNA studies indicates a distant relationship (A. Wolfe, personal communication)...

In studies in Cameroon, Kenfack and Musselman (in preparation) describe "phenotypes" of *S. aspera* collected from areas of different rainfall that could be distinguished on the basis of indumentum, vigor, and corolla color.

In conclusion, the higher significant differences between the two species compared to their low within species variation support the current taxonomic treatment of recognizing two species. It is likely that *S. hermonthica* diverged from its ancestor (most likely *S. aspera*) and with the domestication of cereal crops it was able to evolve strains that parasitized crops. The ability to invade new habitats and hence new hosts corresponded with the development of different morphologies and strains. The necessary genetic changes were made possible through isolation of small demes and finally one of them may have evolved into the taxon now known as *S. hermonthica*. The presence of natural hybrids means that the reproductive isolation is not present and the divergence was recent.

Striga brachycalyx

Herbarium specimens of *Striga brachycalyx*, collected from four localities between latitudes 09 degrees 08' and 13 degrees 08' N, were examined to assess intraspecific variability. *S. brachycalyx* was homogeneous over its range being significantly different in only five variables ($P < 0.002$), three of which were vegetative. The number of significant variables showed a slight increase with latitude especially for sites three and four.

The closely related species *S. aspera* resembles *S. brachycalyx* in overall appearance. They are sympatric in their distribution, frequently in large demes, and occupying relatively drier savannas. Unlike *S. aspera* which occasionally invades the agroecosystem, *S. brachycalyx* is not known to seriously attack food crops although is very common in natural grassland.

Striga gesnerioides Complex

Striga gesnerioides has evolved well known host-specific strains, each parasitizing restricted hosts. Thirteen different variables among seven host-specific strains were studied. The sources of this variation could be due to host plants and/or differences in climatic conditions since the species occupies a wide geographical range.

Generally, strains of *S. gesnerioides* differed significantly in four vegetative and five floral characters ($P < 0.0024$), with the differences between any two strains being less than six variables and averaging two. The strain specific to *Euphorbia* species showed the highest number of significant variables from the remaining strains. It is consistently different from them in its broader leaves (D) and longer corollas (N). The remaining strains showed significant differences in only one or two variables. When compared to each other, strains specific to hosts within the same family (Fabaceae or Convolvulaceae) showed the least number of significant variables. The difference between strains parasitizing Fabaceae was a maximum of 2 with an average of 1 variable, while it was 3 and 1.33 for strains within Convolvulaceae. Not all of the host-specific strains were analyzed with regard to host effects because they were not available from a single site or the same host.

Hosts of *S. gesnerioides* include *Jacquemontia, Merremia*, and *Ipomoea* in the family Convolvulaceae, *Nicotiana* (Solanaceae), *Euphorbia* (Euphorbiaceae), *Vigna, Indigofera* and *Tephrosia* (Fabaceae) with at least four host-specific strains one each of *Euphorbia, Vigna, Nicotiana* and Convolvulaceae (Musselman, 1984; Musselman and Parker, 1981). The *Vigna*-strain is much branched, with a green stem, and bluish flowers causing its greatest damage in the drier regions. The *Euphorbia*-strain has a large single primary haustorium, dark red stem, dark-purple flowers, and is restricted to the drier regions.

The *Nicotiana*-strain is restricted to South Africa and Zimbabwe. It is less branched but otherwise resembles the cowpea-strain. The tobacco strain has not been reported recently and it would be interesting to know if it is still extant. As tobacco is a new world species, the tobacco strain of *S. gesnerioides* may have arisen *in situ*. The strain specific to Convolvulaceae has a slender red and less branched stem with pinkish flowers. The mechanism of host-specificity is not fully understood. Wild (1948) attributed it to germination stimulants; while Parker and Reid (1979) provided experimental evidence that the small flower (*Jacquemontia tamnifolia*) morning glory strain could be germinated by the cowpea root exudate, but not be parasitized.

Musselman and Parker (1981) propose that an incompatibility factor must be responsible for some of the observed specificity. Host specificity has developed under various habitats where the survival strategy is to invade the hosts available in that area, i.e., attacking new hosts in the absence of the conventional hosts. This allowed *S. gesnerioides* to invade new habitats and contributed to its successful spread and abundance throughout almost all Africa, parts of the Arab Peninsula, Asia and the United States of America. Although *S. gesnerioides* has evolved a strong host-specificity among its populations, these "morphs" do not have significant morphological and genetic differences and hence they should be treated as host-specific "strains" of the same species. *Striga gesnerioides* probably evolved from within the species group of *S. lepidagathidis, S. gastonii* and *S. chrysantha* which are confined to natural grassland.

Evolutionary Patterns

This section deals with evolution within the genus, summarizing the cladistic studies presented in Mohamed (1994) and drawing upon our field observations during the past ten years.

As noted above, the first invasion of agroecosystems was probably within the *S. gesnerioides* group. The second was in the species group of S. latericea, *S. forbesii*, and *S. asiatica.* These are likely descended from a species similar to *S. elegans*, and the third was in the Pentapleurae by *S. densiflora, S. curviflora*, and *S. hermonthica.*

Another character that showed homoplasy and reversal was duration. It changed from perennial to annual in *S. chrysantha, S. lutea* and *S. hirsuta* and in all the species in the cladogram above *S. masuria* then was reversed at the node that gave rise to *S. bilabiata* and *S. aequinoctialis* (Figure 1).

When leaf size (character 11), margin (13), and shape (14) were polarized using the Outgroup Methods the plesiomorphic character states were long and broad, serrate margins and 3-nerved, and elliptic or ovate leaves for the three characters respectively. In the same order the apomorphic states were long and narrow or reduced leaves, entire margins, and linear or lanceolate leaves. Results indicate that the three features were reversed and the direction of character evolution was as follows: the reduced and lanceolate leaves with entire margins and the species group of *S. gesnerioides*, the ancestral species at the bottom of the tree) evolved into long, narrow and linear leaves with entire margins in the remaining taxa. An exception was the species on the cladogram between *S. angollii* (Mohamed and Musselman, in preparation) and *S. dalzielii*, and *S. latericea* and *S. forbesii* in which leaves were long and broad, elliptic or obovate, 3-nerved, and serrated margins. This was reversed in *S. linearifolia* with its reduced, lanceolate and entire margins.

In general, all taxa in the Pentapleurae section (defined by character 20) except *S. hallaei, S. angollii*, and the species group of *S. gesnerioides*, shared a common recent ancestor and were clustered at the top of the cladogram between *S. passargei* and *S. aequinoctialis*. *Striga angollii* and *S. hallaei* were unique among the Pentapleurae in having large, elliptic, 3-nerved leaves, with serrate margins. Other features that underwent homoplasy but followed the same path of character polarity were the presence or absence of petioles (character 16), inflorescence density (17), equality of teeth (22), corolla color (23), and corolla tip shape (24).

In all the stages of the analysis using the Lundberg Rooting Procedure, *S. pinnatifida* was fixed as a hypothetical ancestor (where the real outgroup would join the tree) strongly indicating that *Striga* many have evolved from a similar taxon.

It seems likely that *S. gesnerioides* and *S. lepidagathidis* diverged immediately after the divergence of the early *Striga* species such as *S. pinnatifida* and *S. chrysantha* and followed a separate evolutionary line (Figure 1). Data suggest that they share an immediate common ancestor (*S. chrysantha*) that was not shared by the other taxa. They were defined by their ability to parasitize dicotyledons (character 3), succulence (5), and the purple or white stem (7). *Striga lepidagathidis* was defined by the lack of hairs (character 9), including the glabrous corolla tube (25) and acute corolla lobes. *Striga gesnerioides* was separated from these by its ability to attack crops (character 4) and its large haustoria (6).

Striga lutea and *S. hirsuta* shared a single clade and diverged before, and separated from, *S. elegans* and *S. asiatica* by at least five taxa. *Striga lutea* and *S. hirsuta* are partly characterized by their reduced leaves (character 11), sparsely pubescent corollas (25), small bracts (18) and inability to parasitize cultivated crops (4).

The group of taxa between *S. angollii* and *S. asiatica* on the cladogram in Figure 1 shared a recent common ancestor and subsequently diverged into the different species delimited by the features shown at each node. In general, these features included leaf size (character 11), margins and leaf nerving (13), leaf shape (14), pedicels (16), spike density (17), number of ribs (20), and corolla color (23).

The subspecies of *S. bilabiata* and *S. aequinoctialis* constituted the terminal taxa of all *Striga* species and descended from a recent common ancestor. They were characterized by the perennial habit (character 1), acute corolla lobes (24), and cauline leaves (12). The subspecies *S. b. barteri* seems to retain the most ancestral character and the remaining five subspecies of *S. bilabiata* and *S. aequinoctialis* were more likely evolved from a taxon similar to subspecies *S. b. barteri*.

The subspecies *S. b. bilabiata* (the only taxon in this group found outside West and Central Africa) represented the most derived subspecies. The subspecies *S. b. linearifolia (=S. linearifolia)* was an intermediate taxon between *S. b. rowlandii* and *S.*

b. jaegeri supporting its status as a subspecies of *S. bilabiata*. Results also imply a close relationship between the subspecies of *S. bilabiata* and *S. aequinoctialis*. The relationship between these taxa and their position on the cladogram remained constant throughout the entire stages of the program runs.

Among the features used to separate *Striga* from other closely related genera, is the long narrow corolla tube with a distinctive bend just below the limb and the bilabiate corolla. *Striga baumanii* has many features that could be accepted as ancestral that make it a suitable hypothetical rooting for *Striga*. Features include the obscurely bent corolla tube that scarcely exceeds the calyx, and its five similar and almost free corolla lobes. The large seeds of *S. baumannii* strongly mean that it may germinate independent of a host, a pleisomorphic character, but this remains to be confirmed. An unusual feature found only in *S. baumannii* is the indurate, woody corolla; all other species have thin, membranous corollas.

Several authors (Atsatt, 1977; Govier et al., 1968) have emphasized the importance of glandular hairs as excretory organs in hemiparasitic plants. These reduce the high levels of potentially toxic substances emanating from the host (Govier et al., 1968), or play a dual role in some parasitic plants, providing a toxin resistance mechanism and an herbivore repellent device (Levin, 1973). However, *S. baumanii* lacks hairs except for few stiff non-glandular types along the leaf margins.

Perennial *Striga* species apparently have perennial hosts and annual species are associated mostly with annual hosts. Visser (1981) has asked whether an annual parasite induced to grow on a perennial host would assume the perennial habit and if a perennial parasite on an annual host might be able to complete its life cycle fast enough before the host dies off.

With the destruction and disturbances of natural grassland savanna of Africa by humans and livestock, *Striga* species became ruderal and probably tend to favor the naturally disturbed habitats (r-selection) over the more stable habitats (k-selection). The development of annual forms from perennial is of great survival strategy to the parasite and its host and in *Striga* is evident in the short life cycle, with less energy allocated to vegetative growth, and high production of tiny seeds that germinate only under conditions that guarantee the successful establishment of the parasite (e.g. stimulants).

Although species of *Striga* are described as perennial or occasionally perennial (suggested by this study as plesiomorphic) the existence of true perennial forms in most of them is questionable except *S. latericea* (Parker, 1986, 1988), and the host-specific strain of *S. gesnerioides* that parasitize *Euphorbia* (Musselman, 1984). These exceptions are confined to tall perennial grasses where seasonality is less obvious or, in the case of *S. gesnerioides*, to the shrub *Euphorbia* in drier areas.

In the early stages of the diversification of *Striga* species, two separate evolutionary lines may have diverged in different directions. The first evolutionary clade gave rise to *S. chrysantha, S. lepidagathidis* and terminated in *S. gesnerioides*, the most derived taxon in this group. The *S. gesnerioides* species complex (regardless of their evolutionary position among other species) are characterized by many features not common to other members of *Striga*. These include the cespitose growth habit, succulence, reduction of vegetative growth and tendency towards holoparasitism (Thalouarn et al., 1991), the lack of dense hairs or complete glabrosity, and dicotyledon as common hosts.

The second evolutionary line gave rise to the remaining *Striga* species. They are characterized by the stiffly erect growth habit, developed leaves, and hosts which are usually wild grasses or cultivated cereals. Some parasitic plants have a low photosynthetic efficiency compared to non-parasitic members within the same family. Shah et al. (1984) demonstrated that *S. hermonthica* has a low photosynthetic ability and has a high

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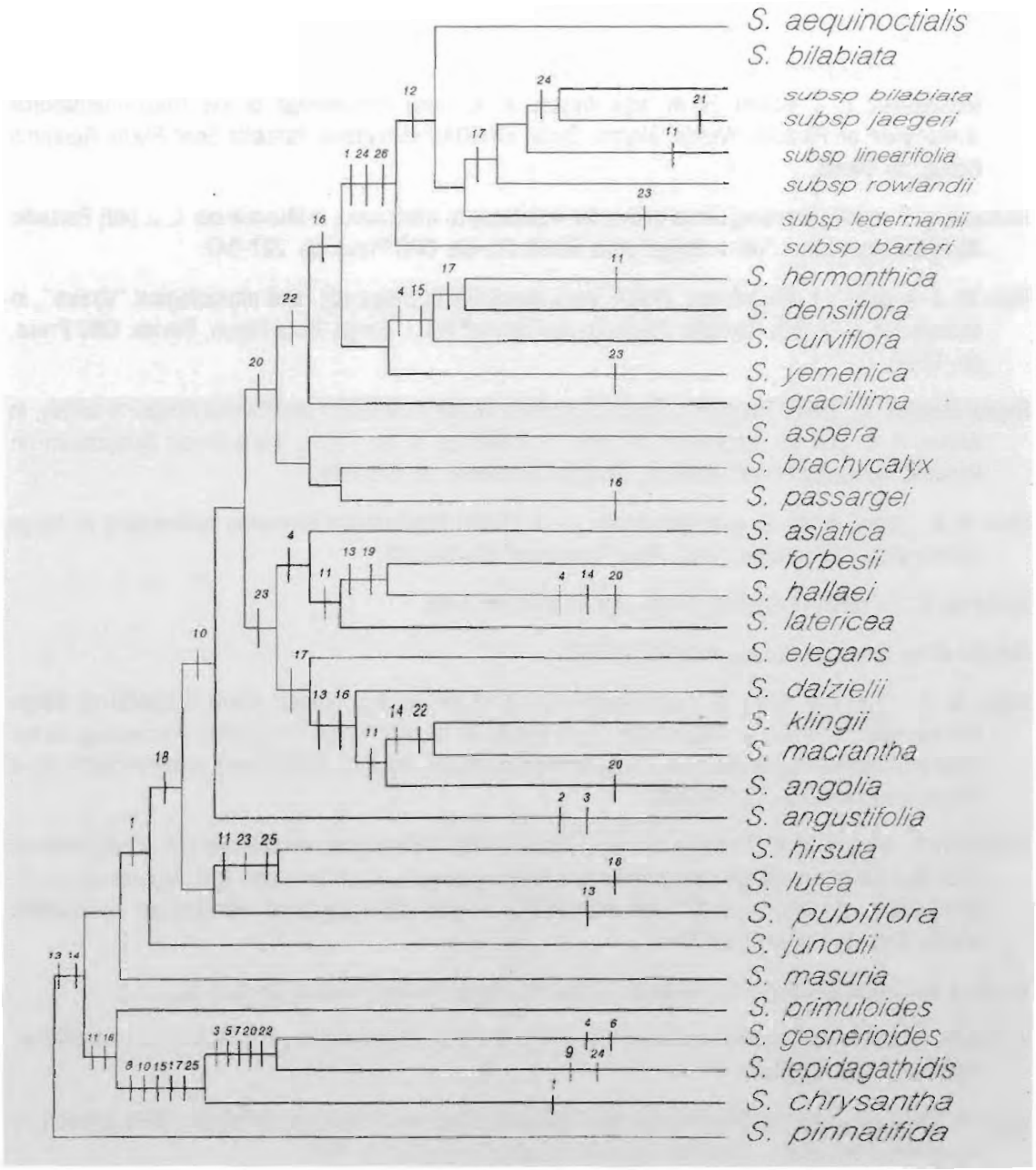

Figure 1

Phylogenetic relationship among menbers of the genus *Striga*.

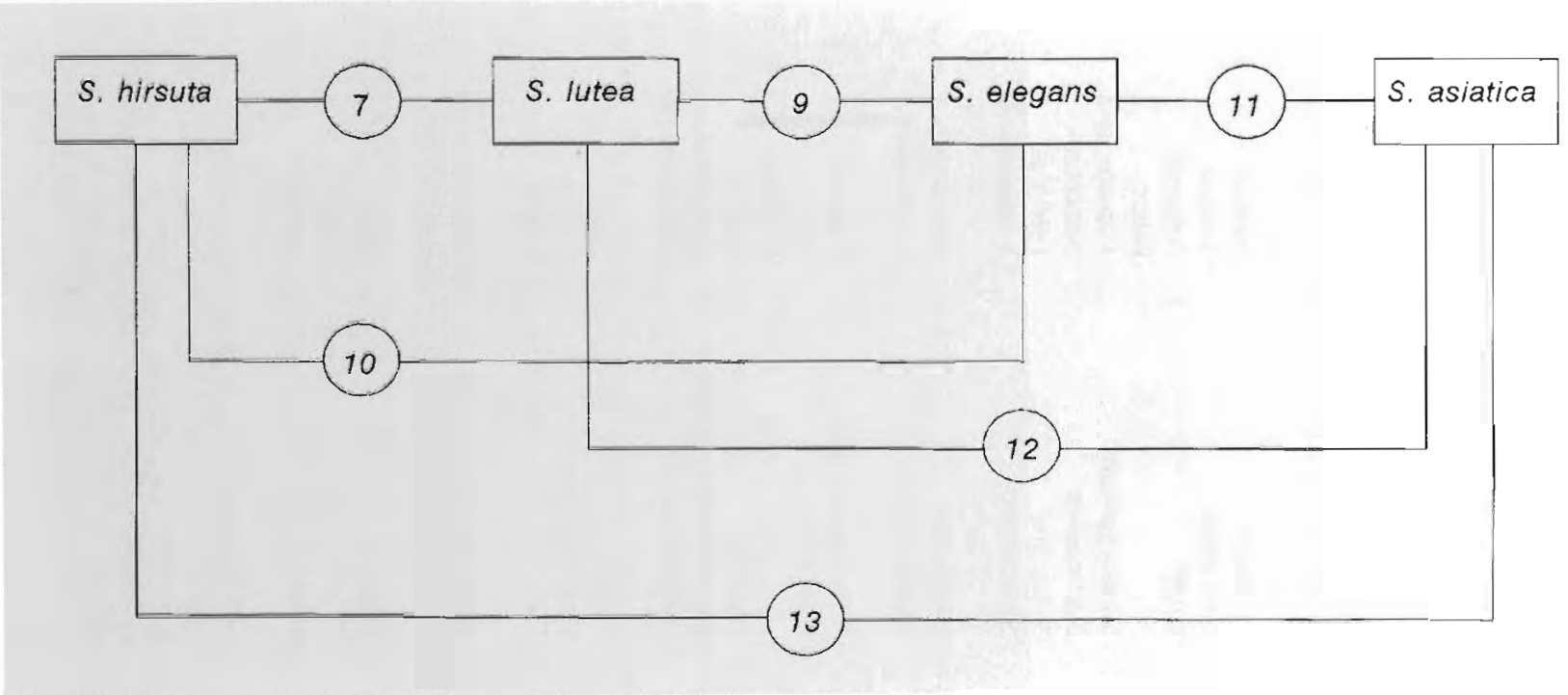

Figure 2

Summary of the variation (in 13 floral and vegetative features) among the species complex of *Striga asiatica*.

Table 1

PLESIOMORPHIC AND APOMORPHIC CHARACTER STATES USED IN PHYLOGENETIC ANALYSIS OF *STRIGA*

CHARACTER	CHARACTER STATE	
	Plesiomorphic (-)	Apomorphic (+)
General:		
1. Duration	0=perennial	1=annual
2. Germination stimulant	0=not needed	1=needed
3. Host specificity	0=none	1=monocot 2=dicot
4. Typical habitat	0=natural grassland	1=agroecosystem
5. Plant succulence	0=not succulent	1=succulent
6. Number& size of Haustoria	0=many small	1=few large
7. Plant color	0=chlorophyllous	1=achlorophyllous
Main stem:		
8. Height	0=stiffly erect	1=cespitose
9. Indumentum	0=glabrous/sparse	1= dense hair
Leaves:		
10. Positon	0=alternate/subalternate	1=opposite
11. Size	0=long broad	1=long narrow 2=reduced
12. caulinenenss	0=acauline	1=cauline
13. Margins & nerving	0=serrate/nerved or pinnatifid	1=entire/not nerved
14. Leaf shape	0=elliptic/ovate	1=linear/lanceolate
Inflorescence:		
15. length	0=short	1=long
16. Flowers pedicellated/sessile	0=shortly pedicellated	1=sessile
17. Flowers dantity	0=lax	1=dense
Bracts:		
18. Length to calyx	0=shorter than calyx	1=longer than calyx
19. Margins & nerving	0=entire/not nerved	1=serrate/nerved
Calyx		
20. Number of ribs	0=10 or more	1=less than 10
21. Teeth number	0=five or more	1=four
22. Equality of teech	0=equal	1=unequal
Corolla:		
23. color	0=purple/blue/ cream/yellow	1=red/pink
24. Lobe tip shape	0=round/obtuse	1=acute
25. Tube pubescence	0=glabrous/sparse	1= pubescent/glandular
26. Corolla bilabiate	0=no	1=yes

Table 2

RESULTS OF CROSSING *STRIGA ASPERA* (SA) AND *S. HERMONTHICA* (SH)

	Female parent		Male parent			Crosses	Capsule formation(%)	Viability (%)	Germination relative %
1.	SA	X	SA			>50	85	34	3250
2.	SH	X	SH			>50	92	38	1100
3.	SA	SH	>50			83	8	600	
4.	SH	X	SA			>50	92	25	2250
5.	#3	(SA X SH)	X	SH		2	50		
6.	#3	(SA X SH)	X	#3	(SA X SH)	5	75		
7.	#3	(SA X SH)	X	#4	(SH X SA)	1	100		
8.	#4	(SH X SA)	X	SA			3	94	
9.	#4	(SH X SA)	X	#3	(SA X SH)	3	83		
10.	SH	X		#3	(SA X SH)	7	88		
11.	SA	X		#3	(SA X SH)	5	85		
12.	SA	X		#4	(SH X SA)	1	0		

Germination was measured over time and relative germination calculated as area under the resulting germination versus time curve.

Introduction

The Orobanchaceae posess many biological features that make them interesting as unique research materials. Among these features are: their origin as holoparasites, their evolutionary pathways, their physiological and anatomical adaptation as obligate parasites, etc. These studies can also have a practical side as new ways for their control as parasitic weeds can be developed.

Although we are surrounded by a "molecular biology" atmosphere, and there is little doubt that the molecular approach can be very powerful in solving taxonomic as well as physiological problems, cytogenetic studies could help in solving many important evolutionary aspects. These include the origin of Orobanchaceae, their evolutionary roots, the origin of temperate *Orobanche* species (most species of this genus grow in cool regions), the origin of the most virulent species parasitizing crops, etc. It would not be logical to abandon a powerful technique just because other powerful technique has been set up, especially when both techniques can complement to each other. But the data are few: in a computerized search, no paper on cytogenetics was found since 1980.

The purpose of the present work is to review and to discuss the cytogenetic studies performed on Orobanchaceae until now as well as to describe the methods to fit their special cytogenetic characteristics. A preview of this subject was given by Cubero and Moreno (1991). The data collected and presented in this chapter could help to raise new interest on the subject. New methodologies, as those based on molecular biology techniques, do not preclude the use of traditional ones; on the contrary, they can complement each other.

Methods and Techniques

Cytological methods and techniques

This section would be unnecessary if not because of the intrinsic difficulties of this material for cytological observation. Most studies were performed on meiosis because the main source of cells for mitotic studies, the root tips, do not exist in Orobanchaceae.

1. *Mitosis*. The few studies published until now on mitosis have used, as favourite material, young ovaries (Hambler, 1956; Greilhuber and Weber, 1975; Weber, 1976a; Palomeque, 1979) and the first pollen grain division (Palomeque, 1979; Weber, 1976a). On occasion petal buds, trichomes (Greilhuber and Weber, 1975) and shoot meristems (Gardé, 1951; Greilhuber and Weber, 1975) have also been used. The first stages of development of the parasite, i.e., the "tubercules", seem not to have been used. However, as a consequence of their fast growth rate, they should be an interesting material because of their very high mitotic index. They are, in fact, an excellent source of DNA for molecular biology studies (Millán et al., 1996).

The most common technique to study the mitosis of young ovaries is very similar to that used for meiotic studies, but Palomeque (1979) modified it because of some difficulties with her material: scarcity of metaphasic cells and a large number of fatty cytoplasmic inclusions that interfered with chromosome separations as well as chromosome observations. This material was not easy for preparations because of its rigidity and its fatty inclusions. Palomeque's technique for young ovaries its summarized as follows:

(1) Only cleaned young ovaries, without any other floral structures, are used. It is useful to cut them in pieces to help chemicals penetrate. A pretreatment with 8-hidroquinoleine 0.002 M, at 0-5ºC for a least 4 hours is recommended.

(2) Wash for 5 minutes with distilled water.

(3) Fixation in glacial acetic acid: absolute alcohol: chloroform (1:3:4) from a least 24h at 0-5ºC.

(4) Wash with distilled water for several minutes.

(5) ClH 1N hydrolysis at 60ºC for 5-7 min., time depending on the kind of material and size of the ovary used.

(6) Stain with acetic orcein 1% for 60 min. at least.

(7) Split the ovary in as small pieces as possible on a drop of acetic orcein 1% on a slide, removing unnecessary rests of material.

(8) Smash the selected material directly on the slide, place the cover glass and complete in the traditional way.

The first division of the pollen nucleus can be studied by the standard technique described by Sharma and Sharma (1972). The steps are:

(1) Anther pretreatement, after cleaning, place in colchicine 0.2% for 1 hour at 18-20ºC wash with distilled water for several minutes.

(2) Anther treatment with 8-hidroxiquinoleine 0.002 M for 1 h at 10-14ºC/WDW.

(3) Fixation in Carnoy for 6 h. at least/WDW.

(4) ClH 1N hydrolisis for 4-5 min., time depending on the material studied, at 60ºC.

(5) Stain with leucobasic fucshine for 2 h.

(6) Squash in 1% acetic carmine.

2. *Meiosis*. Most studies have been performed on pollen mother cells (PMC). There is no physical or biological limitation here as the convenient material, i.e., appropiate anthers, can usually be found over a long period of time. The best material, however, comes from recently emerged shoots. On the other hand, studies on the ovule cytology are very scarce (Carter, 1928; Jensen, 1951; Weber, 1976a).

The technique for meiosis is the traditional one. Some details suitable for this material are referred to below:

(1) The tops of the shoots (best if they are recently emerged from the soil) are fixed in Carnoy (glacial acetic acid: absolute alcohol 1:3, or glacial acetic acid: absolute alcohol: chloroform 1:3:4, in this case mixing just before use). Because of the fleshy broomrape shoot and also because of the large number of other floral organs in the compact broomrape top, it is convenient to use an excess of the fixation reagent, even ten times in volume more than the material to be fixed. It is also convenient to replace it 2-3 times at the very beginning of the fixation process. This removes not only most of the water of the very watery shoot but also eliminates the pigments (especially anthocyanins) present in floral organs and in the stem. The broomrape tops must be fixed in the field. If the plot is far away from the laboratory, a portable refrigerator is very convenient. Then, Carnoy is substituted by 96% alcohol for 1 hour, and then by 70% alcohol. The material can be now stored for 1-2 months or more. Nevertheless, the best slides are produced by recently fixed material.

(2) Staining follows the standard Feulgen or aceto-carmine methods. The hydrolysis time in the Feulgen can change slightly depending on the material. The tops, stored as described above, are placed in glacial acetic acid (saturated with ferric acetate): absolute alcohol 1:3 for 12-24 hours (Palomeque, 1979). After washing with 45% acetic acid for at least 1-2 minutes, the flowers are dissected to obtain the anthers. In 45% acetic acid, smash the anthers directly on the glass, adding a drop of 1% aceto-carmine. The joint action of 45% acetic acid and the aceto-carmine seems to avoid an excessive staining of the cytoplasm (Palomeque, 1979).

(3) Now the squash is completed in the traditional way after placing a coverglass. It the cytoplasm is overstained, a drop of 45% acetic acid diffusing under the coverglass can help. Chromosome staining and maximum contrast are better the next day after staining. The slides

have to be stored in the refrigerator. Hambler (1958) recommends soaking the anthers in concentrated nitric acid for 20 min. in the case of *Cistanche tubulosa*; otherwise, the material cannot be adequately spread on the slide.

Chromosome Numbers

Table 1 updates chromosomes counts on *Orobanche*. There are published counts of only five out of the 17 genera of this family. Most species studied belong to *Orobanche*. However, the knowledge of the chromosome numbers and morphology of other genera would be of great importance to stablish evolutionary trends within the family. For the taxonomy of the American *Orobanche* treatments of Heckard (Heckard, 1973; Heckard and Chuang, 1975) and Thieret (1971) will be followed. For the Old World *Orobanche* the Beck von Managetta (1890, 1907) monographs as well as that of Flora Europaea have been adopted here. Only the most common synonyms are given.

The most thoroughly studied sections (almost 100% of the species), are the American *Gymnocaulis* and *Nothaphyllon*. Two species of *Gymnocaulis* have been studied, especially *O. uniflora* whose interesting problems will be considered below. For *Nothaphyllon* the number and names of species depend on the taxonomic treatment accepted, 10 to 13 according with Munz (1931) or Heckard and Chuang (1975). In any event, it seems that some rare species like *O. Hookeri* Beck von Mannagetta (the only species placed by Beck von Managetta in the *Kopsiopsis* section) or the mexican *O. Dugesii* Munz are still unknown from the cytological point of view; *O. chilense* BvM. was recognized by Beck von Mannagetta to be very close to *O. ludoviciana* Nutt. in this case it would be a synomym of *O. cooperi* Heller according with Heckard (1973). It seems that chromosome numbers of the rest of species have been studied.

The basic number of American species seems to be 2n=48, tetraploidy (2n=4x=96) being common in *Nothaphyllon*. Parthenogenesis is present in *O. uniflora*, and there are probable triploids, arising from crosses between diploids and tetraploids (2n=3x=72), in *O. cooperi*. An atypical count of 2n=38 for *O. ludoviciana* (Löve in Bolkhovskikh et al., 1969), a species according to Munz (1930) but included in *O. cooperi* by Heckard. This could be a misidentified specimen as this number fits in the Old World *Orobanche* section *Orobanche*.

Twenty species of the Old World *Orobanche* were included by Beck von Mannagetta in his section *Trionychon*. This number can be reduced to 15 after eliminating accepted synonyms and with a better knowledge of asiatic species it could be even less. Chromosome counts for four species are known and 2n=24 seems to be well established. A reference of 2n=38 for *O. aegyptiaca* (Srivastava, 1939 in Bolkhovskikh et al., 1969) can be explained by a wrong specimen determination, as that for *O. ludoviciana* referred to above. Among the most important still missing because of their geographical distribution are an Afghan species (*O. orientalis* BvM.) and *O. mongolica* BvM. The comparison with the european *O. oxyloba*, related to them, could throw light on the evolutionary pathways within the genus.

The most important section of the genus, and probably of the whole family, is sect. *Orobanche*, with about 50 species, more than all the rest of the genus. Removing synonymies, 43 species have been considered in the present work; 24 (55%) out of them have been studied belonging to eight out of the nine tribes (89%) originally defined by Beck von Mannagetta (1890). Only the tribe *Amoenae* (with three Central and Eastern Asian species) remains completely unknown from this point of view. For the rest of the tribes, the number of species studied are: *Inflatae* or *Coerulescentes*, 2 out of 6; *Galateae* 4/4; *Curvatae* 6/9; *Arcuatae* 1/3; *Cruentae* 3/4; *Glandulosae* 2/3; *Speciosae* 1/1 and *Minores* 5/12. These are approximations because the taxonomic difficulties of this section.

The basic number is 2n=38, 5 out of 24 species (20%) being tetraploids (2n=4x=76). One species (*O. gracilis* Sm.) shows aneusomaty .

There are also some exceptions. The count of 2n=24 by Zhukov (1939, in Bolkhovskikh, 1969) for *O. cernua* (as *O. cumana* Wallr.) fits *Trionychon* section numbers: it could also be a misidentification. Sugiura (1931, in Bolkhovskikh, 1969) gave 2n=40 for *O. coerulescens* Stephan = *O. ammophyla*), a figure explained by Heckard and Chuang (1975) by a possible wrong count originated by a misinterpretation of the bivalent structure produced by the small size of *Orobanche* chromosomes. But there is a simpler explanation: cytotypes with 40 chromosomes, and even 39, have been seen in *O. crenata, O. minor, O. teucrii* and *O. lutea*. Meiotic anomalies seem to be the rule rather than the exception in many *Orobanche* species when caryological studies are performed at the population level (Moreno et al., 1979). Thus, 2n=40 chromosomes could be a good count for a cytotype of *O. coerulescens*.

The number 2n=40 also characterize *Cistanche* and the only *Conopholis* species known until now. 2n=42 is also seen in one *Cistanche* species and in *Lathrea*; in the latter, 2n= 35 and 36 have been also recorded. The only species of *Phacellantus* studied seems to be a polyploid complex.

It is obvious that we need many more studies in all the genera, including *Orobanche*.

POLYPLOIDY

Likely, all the family has a polyploid origin. The lowest chromosome number recorded is 2n=24, above the limit usual considered for an **ancestral** diploid. But even if we admit that 2n=24 represents an **ancestral** stock (but see next Section), polyploidy plus aneuploidy seems to have played an important role in speciation within the family. Only the section *Trionychon* is different. It is important to mention that in most cases, when a certain *Orobanche* species shows two or more ploidy levels (e.g., 2n=38 and 2n=76), there are not (or not consistent) morphological differences between these cytotypes (Heckard and Chuang, 1975; Weber, 1976b; Moreno et al., 1979; Palomeque, 1979). Two exceptions are found in *O. cooperi*, as there is some variability between cytotypes concerning size and shape of the corolla, and *O. parishi*, the two subspecies being characterized by different chromosome numbers. Very probably the ploidy events, at least in *Orobanche*, are very recent.

If 2n=24 is taken as the ancestral basic number, all sections except *Tryonichon* are characterized by *at least* a chromosome duplication event. A new duplication is also detected in all sections. In this sense, the Orobanchaceae would be excellent material to study polyploidy in action, because all types of polyploidy can be found: auto-, allo- and aneuploidy. As interspecific crosses can be produced between related forms, the difference between allo and autopolyploidy is neither sharp nor very important here. This new cycle of polyploidy is clear in the American species (*O. corymbosa, O. uniflora, O. cooperi, O. parishi*). As mentioned above, the different level of ploidy separates the two *O. parishi* subspecies, *brachyloba* (2n=96) and *parishi* (2n=48).

The Old World species show the same secondary or tertiary cycle of polyploidy. In some cases aneuploidy is an additional source of complication (see next paragraph). This is well documented in the most important section, *Orobanche*. Tetraploidy (relative to 2n=38, actual basic number for the section) has been observed in (*O. densiflora, O. latisquama, O. foetida, O. gracilis* and *O. variegata* (the three latter belong to the same tribe, *cruentae*). Austrian and Spanish cytotypes of *O. gracilis* are characterized by an hexaploid level, i.e. 2n=6x100-120 (Greilhuber and Weber, 1975; Palomeque, 1979), a ploidy level also found in one plant of *O. latisquama* by Palomeque (1979), who also found in *O. rapum-genistae* an even higher level (2n=12x=200-230 chromosomes!!!).

Aneuploidy has been found in *O. gracilis, O. latisquama, O. rapum-genistae, O. crenata* and *O. coerulescens.* In fact, unreduced pollen mother cells can be seen in most cases. Even in *O. ramosa* ssp. *nana* (*Trionychon*), three cells with 2n=42 chromosomes were seen by Palomeque (1979). Three cells may not be significant, but they do reflect the strong tendency for duplication characterizing the whole genus.

A rather surprising fact is that multivalents are rare in both *Orobanche* and *Cistanche* meiosis. Meiosis is very irregular in most species (see below), but trivalents and quadrivalents have been observed only in *C. phelypeae* and *O. rapum-genistae.* This fact **in theoretically diploid material** strongly suggests polyploid ancestors. Among the tetraploids of recent origin (i.e., relative to the actual basic number characterizing the species), *O. densiflora* (2n=76) meiosis does not show multivalents, and *O. gracilis* only shows them in the aneuploid cytotypes, not in its eutetraploids. The very few hexaploid cytotypes analyzed so far have produced very abnormal meiosis, as expected. The small size of the chromosomes can explain the rarity of multivalent associations even in recent polyploids.

Among the American sections, the meiotic behaviour has not been specifically studied, but the plates and comments by Heckard and Chuang (1975) suggest that meiotic abnormalities were only found in a cytotype of *O. cooperi* with 2n=72 as well as in the parthenogenetic race of *O. uniflora* studied by Jensen (1951; see below). Diploidisation seems to be in a very advanced stage in this material.

As Heckard and Chuang (1975) pointed out "...polyploidy is certainly partly responsible for the taxonomic difficulties in the group", a sentence referred to *O. cooperi* but that may be extended to the whole family. Polyploidy, interspecific crosses, hybridisation among different ploidy levels, aneusomaty, parthenogenesis, chaotic meiosis and mitotic abnormalities....: all these facts in the same family cannot be independent as regularity in mitosis and meiosis is genetically tightly controlled. The obvious questions are: What is the cause of such a chaos?, Is it advantageous from an adaptative point of view? We cannot know without a better knowledge of the Orobanchaceae phylogenetic roots. On the other hand, these studies are necessary to discover such phylogenetical roots. This cycle could be broken with the help of molecular biology techniques.

The Basic Number

The number 2n=24 in *Orobanche* was first discovered by Gardé (1951). He suggested n=6 as the basic number instead of n=19, until then the only known number. *Orobanche ramosa* (and then *Trionychon*) would have originally been tetraploid (2n=4x=24); the other two species studied by him, *O. crenata* and *Cistanche* spp. would be heteroploids (6n+2 and 6n+4 respectively).

As 2n=12 (n=6) has not yet been found in the family, Heckard and Chuang (1975) suggested a polyploid series with x=12 as the basic number. The two American sections (2n=48) fit well in this hypothesis, but the count 2n=36 for *O. uniflora* ssp. *uniflora* does not. As this species is parthenogenetic, the reason for this number could be found in a common process leading both to parthenogenesis and to a different chromosome number. The cytotype with 36 chromosomes could have been produced within a population with 2n=24 chromosomes by unreduced ovules (n=24) fertilized by normal pollen (n=12). Such a mechanism is known in Solanaceae, for example. But this hypothesis would require a very fast and efficient process of diploidization of a triploid with 36 chromosomes. Alternatively, this problematic cytotype could have originated by a cross between two populations with 48 and 24 chromosomes, the latter still to be discovered in America. A simpler explanation was proposed by Heckard and Chuang (1975); they suggested that the 2n=36 was a wrong number (they had recorded 2n=48 on *O. uniflora* ssp. *occidentalis*), the mistake being likely produced by six pairs of small chromosomes. A

common process leading to parthenogenesis and polyploidy, very well known in angiosperms, is the most acceptable explanation with the known data.

Thus, 2n=24, found in the section *Trionychon* of *Orobanche*, would represent the ancestral diploid number according to Heckard and Chuang (1975). Section *Orobanche* would be a secondary poliploid. But 2n=24 (n=12) is a rather large chromosome number for an **ancestral** diploid; n=6 seems to me a more likely **original** basic number. An obvious tendency towards polyploidy in *Orobanche* as well as in the rest of Orobanchaceae genera was described in the previous paragraph. Besides, the Orobanchaceae may be ancient parasites and this habit can favour a lack of strict genetic control of meiosis. Finally, the lack of species with 2n=12, is not a proof that n=6 is not the true ancestral basic number. Many cases are known of families with a low ancestral chromosome number lost or almost lost in present day family representatives. One of these is the Fabaceae, with n=7 as well stablished ancestral number almost lost at the present time (only shown in *Cercis* among the primitive legumes), the *actual* basic being rather 2n=28. The Fabaceae, as the Orobanchaceae, also show many cases of secondary polyploidy (Goldblatt, 1981). It is not surprising: the evolutionary mechanisms are essentially the same.

The evolutionary events in the Orobanchaceae can be summarised as follows (Fig.1):

(1) The **original** basic number would have been x=6 (2n=12); a very old duplication led to 2n=4x=24. A process of diploidization produced a stable chromosome complement in a part of the genus *Orobanche* (sect. *Trionychon*) which contains the lowest **actual** basic number.

(2) A second duplication happened producing 2n=48 forms (octoploid or tetraploid depending on the reference basic number, the ancestral or the new one). Some taxa stabilized it, as sections *Gymnocaulis* and *Nothaphyllon*, the American native representatives of *Orobanche*, "parental" species with 2n=24 being lost or undiscovered.

(3) The second duplication did not follow a process of diploidization in many other groups of the Orobanchaceae. Rather, aneuploidy led to different chromosome numbers which finally were stabilized (secondary polyploidy). In this way, 2n=38 (section *Orobanche*), 40 (*Cistanche*, *Conopholis*), 36 or 42 (*Lathraea*) were finally settled, as well as some others (e.g., 2n=42 in *Cistanche*, 36 in *O. uniflora*).

(4) There is still a tendency to produce new duplications. *Phacellanthus tubiflorus* is, in fact, a giant polyploid complex, containing many different numbers (from 2n=38 to 84). Several species belonging to section *Orobanche* present 2n=76, cytotypes (sometimes at the subspecific level) with 2n=96 are found in the American sections of *Orobanche*, aneuploids are very frequent in most populations of *Orobanche*. In some rare cases, the process of chromosome duplication seems to be out of control, the number of chromosomes exceeding 100 and even 200, as in some cytotypes of *O. gracilis* and of *O. rapum-genistae* respectively.

(5) In some cases, polyploidy is combined with other biological features as parthenogenesis and aneusomaty, also known in other families with strong polyploid tendencies.

Meiotic Behaviour

Meiotic abnormalities are expected in a polyploid material, especially if they are of recent origin. However, meiotic behaviour has stimulated little interest. Most studies have dealt with simple chromosome counts, although they only provide a part of the information (and not always the most important) that can be obtained from caryological studies. Table 2 summarize the available data.

All possible meiotic anomalies are found: anaphasic bridges, uni-, tri- or quadrivalents and secondary associations, lagging chromosomes, complete failure of the meiotic process, erroneous cell wall formation in dyads or tetrads, etc. (Moreno et al., 1979; Palomeque, 1979; statistics of these data are not shown in Table 2.) The very frequent association between bivalents, as well as the tendency to chromosome agglutination (shown by *O. purpurea* and *O. elatior*) again strongly suggests a polyploid origin.

There are only three species with a completely regular meiotic behaviour, but very likely this result cannot be taken as definitive as in all three the number of PMC observed was very small (6, 15 and 30). On the other hand, the specimens with a very high chromosome number are characterized by very abnormal meiosis, as expected, reaching a total abnormality in some cytotypes of *O. gracilis* and *O. rapum-genistae*.

Table 2 shows a *tendency* of the species studied to present a greater number of anomalies in the first meiotic division. This fact is clear when comparing sections *Trionychon* and *Orobanche*, the former only showing anomalies in the first meiotic division. It is not a characteristics separating both sections as *O. loricata* also shows that feature. The two *Cistanche* species studied also show the same difference, but obviously many more studies are required in order to establish sound conclusions.

The general picture shown by the results in Table 2 is a polyploid origin even for those species with a less irregular meiosis (e.g., *C. violacea*, *O. ramosa*, *O. loricata*) which could be undergoing a process of diploidization (the three species with aparently regular meiosis are excluded from this comment for the reason given above).

Pollen Fertility

A direct consequence of the meiotic abnormalities could be a loss of pollen fertility. The usual technique to evaluate this is to stain the pollen grains with aceto-carmine to avoid the difficulties of a true "in vitro" test. Although there are several sources of error, most authors accept that stained and normally shaped pollen grains are fertile or, at least, that their proportion is well correlated with that of the functional pollen.

The relationship between pollen fertility and meiotic abnormalities must be very carefully evaluated, because an irregular meiosis is *not* the only source of pollen sterility. The correlation coefficient between pollen sterility and percentage of meiotic abnormalities, calculated by Palomeque (1979) was 0.73 ($p < 0.01$, $n=28$). But neither the most perfect meiosis yielded 100% fertile pollen nor the only case she registered of 100% meiotic abnormalities produced fully sterile pollen (the actual figure was a 60%). Thus, the meiotic behaviour of the Orobanchaceae so far studied suggests an effect on the loss of the pollen fertility but it does not explain all this loss. Even with only a small percentage of fertile pollen grains the reproductive potential of the Orobanchaceae is still inmense.

There are no studies on the ovule fertility, but cytogeneticists think that the egg cell is much better buffered than the pollen against chromosomic unbalanced structures. Thus, the limiting factor in Orobanchaceae would only be the male gamete, and as it has been seen, the maximum proportion of pollen sterility occurred in a material showing a 100% of irregular meiosis, and that proportion was only 60%.

Chromosome Characteristics: The Mitotic Chromosome

Mitotic studies, which are necessary to establish the main morphological characteristics of the chromosomes, are particularly difficult in Orobanchaceae. This is why chromosome descriptions are far less in the literature than counts in this familiy. Palomeque (1979) performed a study on several species of *Cistanche*

and *Orobanche* providing the only available description of these caryotypes. Table 3 summarizes the main findings. The smallness of the chromosomes of this family and the consequent lack of accuracy of the chromosome measurements (especially in the species with a high chromosomic number) are further difficulties in obtaining significant results. The present discussion is limited by these constraints.

1. Position of the centromere

All species described in Table 3 show a high proportion of subtelocentric (st) and submetacentric (sm) chromosomes, metacentric (m) chromosomes being very rare, especially in *Orobanche*. Metacentrics are completely lacking in *O.ramosa* and *O. purpurea* (sect. *Trionychon*) and they are much more frequent in *Cistanche* than in *Orobanche*. Subtelocentric chromosomes are almost absent in *Cistanche*. The distinction among the different chromosome types is obscured by the difficulties in precise measurements. The largest values of the arm ratios is 4.3 in *Orobanche* (*O. cernua*) and 3.5 in *Cistanche*.

There is not any clear relationship among the caryotypes of *Orobanche* and *Cistanche*. The "average" centromere is in the submedian region (arm ratio average = 1.7) in *Cistanche* and it produces a typical submetacentric chromosome in *Orobanche* (arm ratio averages 2.1 and 2.2 in *Trionychon* and *Orobanche* respectively). In other words, the "average" chromosome is somewhat more symmetrical in *Cistanche* that in *Orobanche*.

The relationships between the caryotypes of the two *Orobanche* sections are not clear either. None of the four species studied belonging to section *Orobanche* show a proportion of m:sm chromosomes similar to the 3:7 ratio present in section *Trionychon*. This proportionality could have been found if polyploidy (including aneuploidy) were the *only* factor in *Orobanche* evolution. In other words, had section *Orobanche* evolved from an ancestor as a consequence of *only* polyploidy, and had the loss of chromosomes by aneuploidy been at random, the chromosome morphology of both sections *Trionychon* and *Orobanche* would have been very similar. But this does not seem to be the case as available data show.

These facts suggest that not only polyploidy and aneuploidy but also chromosomic structural rearrangements have occurred. The morphology of the chromosomes with secondary constriction support this hypothesis. In *O. ramosa* this is a subtelocentric chromosome, while in both *O. cernua* and *O. minor* this chromosome is metacentric or submetacentric. The presence of a heteromorphic pair of chromosomes in *O. cernua* can also be explained by structural rearrangements. Greilhuber and Weber (1975) also suggested the possibility of structural arrangements in *O. gracilis* because of the presence of so many chromatin bridges in anaphase I. That explanation is also valid for *O. crenata* (Moreno *et al.*, 1979). The difference between the two *C. phelypaea* specimens studied could also be explained by minor structural differences.

2. Chromosome size

With the exception of *O. cernua*, the species of the two sections of *Orobanche* studied show very similar chromosome lengths (mean values of 2.6-3.5). The "average" chromosome is about 3 long, a very small size. *Cistanche* chromosomes are different, roughly being at least double the size of those of *Orobanche*. The total length of its haploid complement (n=20) is obviously much larger than that of *Orobanche*, averaging 140.

These results reveal again a difference in chromosomic structure, in this case between genera. It is interesting to compare the mean size of the chromosome. *Cistanche* and *O. cernua* show the greater medium chromosome size, roughly double than that of the rest of *Orobanche* species. Two explanations can be offered:

(a) The *Cistanche* primitive chromosome is derived from a smaller one (similar to that of *Orobanche*) by tamden duplications or endomitosis.

(b) The *Cistanche* chromosome is closer to the primitive one than that of *Orobanche*; in this case, the latter would have been the consequence of chromatin loss during its evolution.

If the data for *O. cernua* are confirmed (measurements were taken in this case *only* in young ovaries), the easiest way to explain this difference is, as in other genera, through a loss of chromatin, possible in certain circumstances as, for example, redundant DNA. Thus, the original chromosome of the family would have been long (perhaps around 10-12?) and succesive genomic rearrangements would have produced a loss of chromatin compensated by an increase in the number of chromosomes by polyploidy. A certain proof of his suggestion is given by the chromosome size of a cytotype of *O. rapum-genistae* showing the maximum number of chromosomes recorded in the Orobanchaceae (n>100). Palomeque (1979) was not able to measure them accurately, but their medium sizes varied between 0.3 and 1.0 , thus, number and size seem to be in an inverse relation. Greilhuber and Weber (1975) were not able either to describe the chromosome morphology of polyploids.

Orobanche would be, in this way, a more evolved genus than *Cistanche*.

3. Asymmetry of the haploid complement

Three indices are presented on Table 3. The value r is the averaged ratio betwen chromosome arms (large/short); *Cistanche* chromosomes show the greatest similarity between arms: the averaged chromosome is clearly submetacentric. *Orobanche* chromosomes have more diverse chromosomic arms, especially sect. *Trionychon*, its largest arm being, on the average, more than twice the short one. The ratio largest/smallest chromosome (z) is around 2 for *Cistanche*, a little lesser than 2, on the average, for sect. *Trionychon* and clearly larger than 2 for sect. *Orobanche*. Finally, the similarity index s measures the proportion (in %) of chromosomes with $r \leq 2$. *Cistanche* chromosomes are very similar to each other, 80-85% of them belong to the same architectural type whereas *Orobanche* sect. *Trionychon* chromosomes are more irregular, the index being, on the average, close to 40%. Finally, *Orobanche* sect. *Orobanche* shows itself again to be more variable than the two other taxa. Even removing *O. cernua*, its similarity index varies betweeen 50% and 100%.

If genomes with more or less similar chromosome lengths are primitive, as seems to be the rule, sect. *Orobanche* is obviously derived from others, probably through chromosomic rearrangements at it was suggested above. *Cistanche* retains some primitive features in its chromosomes.

Three Study Cases

In this section three cases are presented to show how the variability in chromosomic, genomic and caryotypic structure can be extremely large when in-depth studies are performed. Parthenogenesis in *O. uniflora*, aneusomaty in *O. gracilis* and the meiotic behaviour of *O. crenata* populations are presented.

1. Parthenogenesis in *O. uniflora*

According to Jensen (1951), the first notice of the meiotic behaviour of *O. uniflora* is credited to Jeffrey, who never published the findings of his studies, which started at least in 1927. Jensen, under Jeffrey's supervision, was able to compare preparations made in 1927, 1932 and again in 1947. All this material was collected in the vicinity of Cambridge, eastern Massachussets.

The finding that set up the study of meiosis was that castrated flowers were able to produce seeds.

giant nuclei, and metaphase I with chromosomes out of the plate. Very frequent, but not present in all the morphological types, were collapsed anaphase I and bridges in anaphase I. Present only in some types were bridges in anaphase II and cytokinesis not synchronized with caryokinesis. But it was impossible to find any relationship between these meiotic features and the morphological ones.

Moreno et al., (1979) suggested several possible explanations for this meiotic behaviour. First of all, *O. crenata* could be derived from an interspecific (or intersubspecific) hybrid. It is not difficult to find a similar behaviour in many other interspecific hybrids not related at all with *Orobanche*. Hybridization is more likely to occur within a polyploid complex than between diploid species. The polyploid nature of *Orobanche*, and perhaps of all Orobanchaceae, seems to be well established after the results discussed in the present chapter. Thus, it could be that the genus *Orobanche* was formed by several polyploid complexes probably overlapping to a certain extent. A second explanation was aneusomaty. It is clear that aneusomaty can disturb the correct meiotic behaviour (see above), but it would be necessary to demonstrate the general occurrence of aneusomaty in the genus.

In any event, the widespread meiotic abnormalities in *O. crenata* can only be a reflection of the situation in *Orobanche* as well and likely in most of the Orobanchaceae (Table 2). Moreno et al (1979) also found these anomalies in *O. minor* and *O. medicaginis* although they did not conducted a detailed study. It seems that both the genus and perhaps the entire family are under very active and rapid evolutionary processes. New forms, new races, new genic wide interchanges can be continuously produced. Some of these forms can become very agressive parasitic weeds.

Concluding Remarks

The preceding pages have shown that traditional studies can throw light on important evolutionary problems even in so complicated family as the Orobanchaceae. Figure 1 shows a hypothetical phylogenetic tree based on these findings. This tree is very incomplete, as there is only one well studied genus (*Orobanche*) and many genera still are completely unknown to the cytogeneticists. As stated above, the family seems to be in an active process of evolution as demonstrated by the chromosome instability in several aspects: the basic number, the total genomic length, the individual chromosome size and morphology, the meiotic irregularity without substantially affecting the pollen fertility, etc. What is the reason for maintaining such a "good health" against the paradigm of the required chromosomic and genomic stability?.

The general impression is that the whole family is composed of different polyploid complexes. It is well known that a polyploid complex is a permanent source of new genomic combinations, i.e., new potential species. Are there new parasitic species "on the pipeline"? Is this the origin of the most aggresive broomrapes? Could we prevent the formation of new virulent races and species by breaking the gene flow among species adapted to different ecological regions?

The ancestral chromosome was likely long i.e., *Cistanche*-like or longer, and there is in the material known so far an obvious trend to increase the chromosome number while reducing the individual chromosome size. This fact is dramatically exemplified by the comparison between two cytotypes of *O. rapum-genistae*, the "standard" one with n=19 and the other one with n>100: the chromosomes of the latter proved to be of very difficult study as their lengths varied between 0.3 and 1.0. Can we correlate this evolutionary pathway with parasitic behaviour? Does the loss of chromosomic material mean that the parasite is getting rid of useless genes for parasitism? Does the increase in number of smaller chromosomes mean that the parasite is producing more copies of useful genes for parasitism? How are these used by the parasite for a better attachment to the host, a better removal of nutrients, efficient reproduction...?

There are many fields, as those outlined above, that produce fruitful cooperation among different disciplines: histology, physiology, ecology, systematics, cytogenetics and many others by use of traditional techniques and the molecular biology methods. Difficult problems, as parasitism is, require complex approaches.

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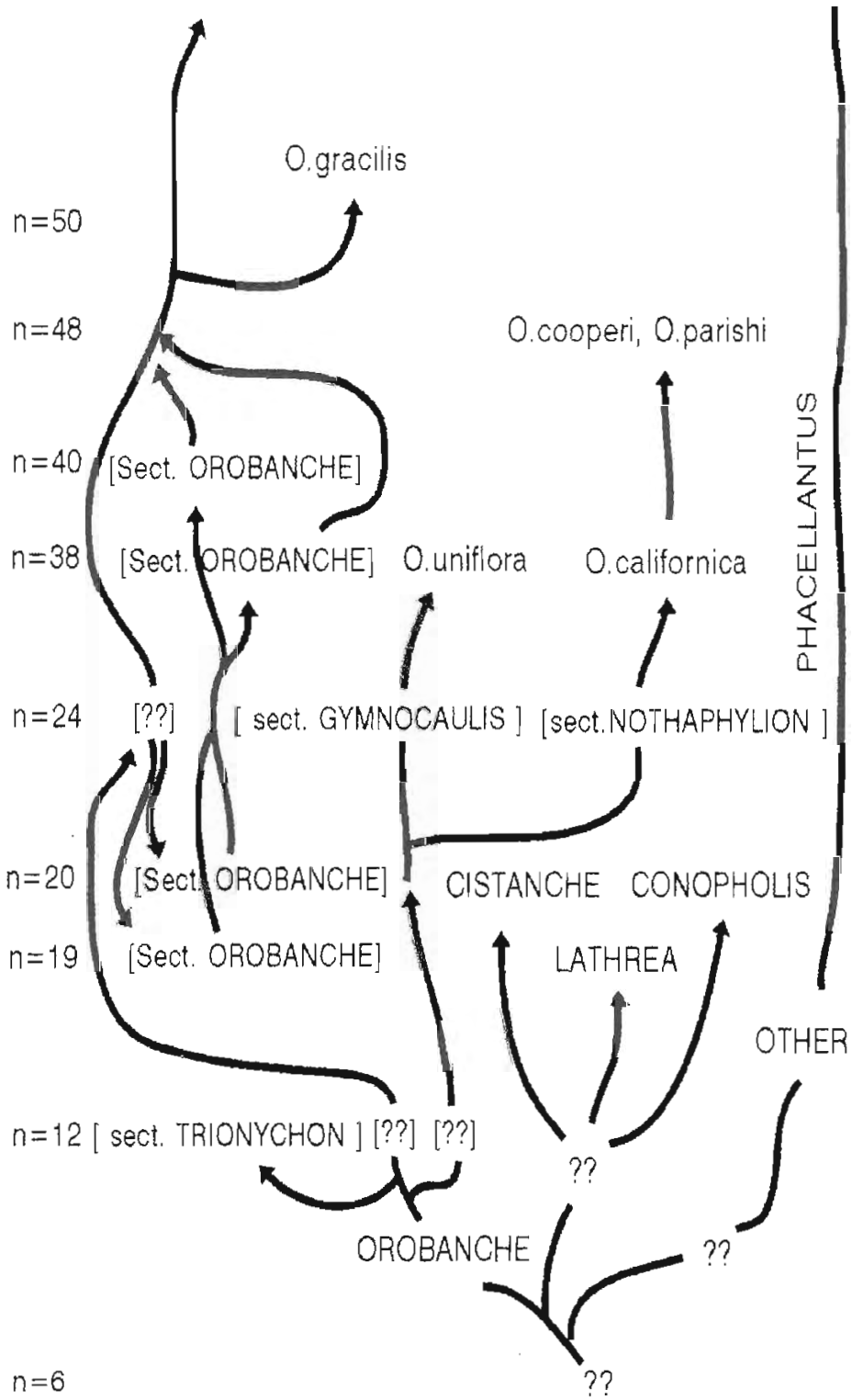

Figure 1

Cytological relationships in Orobanchaceae.

Table 1

CHROMOSOME NUMBERS IN OROBANCHACEAE

	2n	Observations	Authority
CISTANCHE			
C. phelypaea P. Cout.	40	PMC, stem	Gardé 1952
ssp. *lutea* Fdez. Casas et Lainz	40	PMC, pollen	Palomeque 1979
C. tinctoria G. Beck	42	probably PMC	Kadry in B
C. tubulosa (Schenk.) Wight	40	PMC	Reese in B
	40	PMC	Hambler 1956, 1958
C. violacea (Desf.) G. Beck	40	PMC, pollen	Palomeque 1979
CONOPHOLIS			
C. americana Wallr.	40	probably PMC	Lewis in B
LATHRAEA			
L. clandestina L.	42	probably PMC	several authors in B
L. squamaria L.	35	probably PMC	Sorsa in B
	36	probably PMC	Witrch in B
	36,42	probably PMC	Tischler in D & W
	42	probably PMC	Gates, Latter in B
PHACELLANTHUS			
P. tubiflorus Sieb. et Zuck.	38,57,76	probably PMC	Matsuura and Toyohuku in D&W
	42,70,84	probably PMC	Matsuura in B
OROBANCHE			
Section *Trionychon* Wallr.			
O. aegyptiaca Pers.	24	probably PMC	Zhukov in B
	38	probably PMC	Srivastava in D & W
O. caesia Reich.	24	Stem	Greilhuber & Weber 1975
O. purpurea Jacq.	24	PMC, ovaries	Hambler, 1956, 1958
	24	PMC, pollen	Palomeque, 1959
O. ramosa L.	24	PMC	Gardé 1952, Hambler 1956,1958
ssp. *ramosa*	24	PMC, pollen	Palomeque 1979
ssp. *mutelii* Cout.	24	PMC, pollen	Palomeque 1979
ssp. *nana* Cout.	24	PMC, pollen	Palomeque 1979
Section *Orobanche* (=*Osproleon* Wallr.)			
O. alba Steph. (=*O. epithymum* D.C.)	38	PMC, ovaries	Weber 1976
O. alsatica Kirsch.	38	PMC, MMC, ov.	Weber 1976
O. amethystea Thuill.	38	MMC, ovaries	Weber 1976

Table 1 ***(Cont.)***

CHROMOSOME NUMBERS IN OROBANCHACEAE

	2n	Observations	Authority
	38	PMC	Delay and Petit 1971
ssp. *amethystea*	38	PMC	Palomeque 1979
O. caryophylacea Sm.	38	MMC, ovaries	Weber 1976a
(=*O. vulgaris* Poir.)	38	PMC	Hambler 1956, 1958
	38	PMC	Palomeque 1979
O. cernua L.	24	probably PMC	Zhukov in B
(*O. cumana* Wallr.)	38	PMC	Hambler 1956, 1958
	38	PMC, pollen,ov.	Palomeque 1979
O. coerulescens Steph. ex Willd.	38	Ovaries	Weber 1976a
(=*O. ammophyla*)	38	probably PMC	Matsuura in B
	40	probably PMC	Sigiura in B
O. crenata Forsk.	38	PMC	Gardé 1951
(=*O. speciosa* D.C.)	38	PMC	Moreno et al. 1979
	38,39,40	PMC	Palomeque 1979
O. densiflora Salzm.	76	PMC	Delay and Petit 1971
	76	PMC	Palomeque 1979
O. elatior Sutton	38	Ovaries	Weber 1976a
(=*O. major* L. p.p.)	38	PMC	Hambler 1956, 1958
	38	PMC	Palomeque 1979
O. flava Martins	38	probably PMC	Skalinska et al. in B
	38	PMC, ovaries	Weber 1976
O. foetida Poiret	76	PMC	Palomeque 1979
O. gracilis Sm.	76,78,80,82	PMC	Palomeque 1979
(=*O. cruenta* Bertol.)	76,100,120	Pollen	Palomeque 1979
	76(73-91)	PMC, ov., stem	Palomeque 1979
	114 (112-116)	trichomes	Greilhuber and Weber 1975
O.hederae Duby	38	ovaries	Weber 1976a
	38	PMC	Hambler 1956, 1958
	38	PMC	Palomeque 1979
O. laserpitii-sileris Reuter ex Jordan	38	PMC,MMC,ovaries	Weber 1976a
O. latisquama Balt.	76	PMC,pollen,ov.	Palomeque 1979
	82-86,76-79	PMC, ovaries	Palomeque 1979
O. loricata Reich.	38	Ovaries	Hambler, 1956, 1958
(incl.*O. picridis* F.W. Schultz)	38	MMC, ovaries	Weber 1976a
O. lucorum Braun	38	probably PMC	Palmgren in D & W
O. lutea Baumg.	38	probably PMC	Zhukov in B
(=*O. medicaginis* Baum.)	38	MMC,pollen,ov.	Weber 1976a
O. minor Sm.	38	MMC	Carter 1928
	38	PMC	Hambler 1956
	38	probably PMC	Love in B
	38	PMC,pollen	Palomeque 1979

Table 1 *(Cont.)*

CHROMOSOME NUMBERS IN OROBANCHACEAE

	2n	Observations	Authority
	38	ovaries	Weber 1976a
(as *O. maritima* Pugsley)	38	PMC	Hambler, 1956, 1958
O. rapum-genistae Thuill.	38	PMC	Hambler, 1956, 1958
(=*O. major* p.p.)	38	PMC,pollen,ov.	Palomeque 1979
	38->200	Pollen	Palomeque 1979
O. reticulata Wallr.	38	ovaries	Weber 1976a
	38	probably PMC	Favarger in D & W
	38	PMC	Hambler, 1956, 1958
O. salviae F.W. Schultz	38	PMC,ovaries	Weber 1976a
O. teucrii Holandre	38	PMC,ovaries	Weber 1976a
O. variegata Wallr.	76	MMC,pollen	Weber 1976b
Section *Gymmocaulis* Nutt.			
(=*Aphyllon* G. Beck)			
(=*Euanoplon* Thieret)			
O. uniflora L.			
ssp. *uniflora*	36,72	PMC,MMC,ov.	Jensen 1951
ssp. *occidentalis* Ferris	48	PMC	Heckard & Chuang 1975
O. fasciculata Nutt.	48	PMC	Heckard & Chuang 1975
Section *Nothaphyllon* Heckard			
(=*Myzorrhyza* Beck)			
O. bulbosa G. Beck	48	PMC	Heckard & Chuang 1975
O. californica Cham. & Schl. (5 ssp)	48	PMC	Heckard & Chuang 1975
(*O. grayanaka* Beck)	48	probably PMC	Anderson in B
O. cooperi Heller	48,72,96	probably PMC	Heckard & Chuang 1975
(*O. multicaulis* Ferris)			
(as *O. ludiviciana* Nutt.)	48,96	probably PMC	Heckard & Chuang 1975
	38	probably PMC	Löve in B
O. corymbosa Ferris	48,96	PMC	Heckard & Chuang 1975
O. parishii Heckard			
ssp. *parishii*	48	PMC	Heckard & Chuang 1975
ssp. *brachyloba* Heckard	96	PMC	Heckard & Chuang 1975
O. pinorum Geyer	48	PMC	Heckard & Chuang 1975
O. valida Jeps.	48	PMC	Heckard & Chuang 1975
O. vallicola Heckard	48	PMC	Heckard & Chuang 1975

PMC and MMC: pollen and megaspore mother, cells. Counts in meiosis, prometaphase I. All the other counts are made in mitosis of somatic tissues or pollen mitosis.

***B:** BOLKHOVSKOKH et al. (1969)*
***D & W:** DARKINGTON AND WYLIE (1955)*
***W:** WEBER, 1976*

Table 2

MEIOTIC ANOMALIES IN OROBANCHACEAE (1)
(% ANOMALIES IN POLLEN MOTHER CELLS) (2)

	2n	I:d-m	I:m-t	II	total
CISTANCHE					
C. phelypaea	40	12.0	36.4	41.2	23.5
ssp. *lutea*	40	8.3	11.8	9.1	9.9
C. violacea	40	6.3	0	0	2.0
OROBANCHE					
Section *Trionychon*					
O. ramosa					
ssp. *ramosa*	24	13.0	11.8	0	5.9
ssp. *mutelii*	24	15.4	12.8	0	8.9
ssp. *nana*	24	23.1	10.0	0	9.4
Section *Orobanche*					
O. amethystea					
ssp.*amethystea*	38	31.8	23.1	6.3	21.5
O.caryoph.(3)	38	0	0	0	0
O. cernua	38	41.3	16.9	1.8	23.7
O. crenata	38	18.9	9.1	7.1	10.0
	38-40	17.4	22.2	30.2	21.0
O. densiflora	76	41.0	10.7	5.0	20.4
O. elatior(4)	38	0	0	0	0
O. foetida	76	62.7	25.0	25.0	36.5
O. gracilis	76	35.5	22.2	23.5	25.4
	100-120	100	100	76.9	94.4
	73-91	10.6	38.9	44.4	41.5
O.hederae(5)	38	0	0	0	0
O. loricata	38	16.7	0	0	6.9
O. minor	38	16.7	23.1	9.1	13.0
O. rapum-gen.	38	43.9	18.5	18.8	30.0
	>200	100	100	100	100

Observations:
(1) Recalculated from data from Palomeque (1979)
(2) I, II: first and second meiotic divisions.
d, m, t: respectively: diakinesis, metaphase, telophase.
(3)(4)(5): respectively 15, 6 and 30 pollen mother cells only

Table 3

MITOTIC CHROMOSOMES IN OROBANCHACEAE (1)(2)

	n	h	ave.(min.max)	M	m	sm	st	SAT	r	s	z
CISTANCHE											
C. phelypaea											
ssp. *lutea*	20	134.0	6.7(4.9-9.6)	2	11	6	1	1	1.6	80	2.0
ssp. *lutea*	20	178.5	8.9(6.3-12.4)	3	10	7	0	-	1.5	85	2.1
c. violacea	20	127.5	6.4(4.6-9.9)	3	10	7	0	-	1.5	80	2.0
OROBANCHE											
Section *Trionychon*											
O. ramosa											
ssp. *ramosa*	12	35.2	2.9(2.5-3.9)	0	3	7	2	-	2.3	42	1.6
ssp. *mutelii*	12	35.8	3.0(2.5-3.9)	0	3	7	2	1	2.4	25	1.8
ssp. *nana*	12	42.0	3.5(2.4-5.1)	0	3	7	2	1	2.2	42	2.1
O. purpurea	12	31.0	2.6(1.9-3.4)	0	3	7	2	-	2.3	42	1.8
Section *Orobanche*											
O. cernua(*)	19	111.2	5.9(4.0-8.7)	1	9	4	5	1	2.0	58	2.2
O. latisquama(**)	38	127.6	3.3(1.4-5.9)	0	22	12	4	-	3	71	4.2
O. minor	19	62.2	3.3(1.8-4.8)	1	8	8	2	1	1.9	53	2.7
O. rapum-gen.(**)	19	58.9	3.1(1.9-5.4)	0	9	9	1	-	1.7	95	2.8
		>100	0.3-1.0 aprox.								

(1) Prepared from data from Palomeque (1979)
Observations on first mitosis of the pollen grain except:
(*) *only* on young ovaries
(**) *also* on youn ovaries

(2) **n**: haploid complement: total length of the haploid complement in μ
ave: average chromosome in μ
min-max: range of chromosome lenght variation in μ
M-m-sm-st: chromosome type according to the large/short arm ratio, respectively 1.0, 1.0-1.7, 1.7-3.0, >3.0
SAT: chromosome with secondary constriction.
r: average large/small chromosome ratio
s: % of chromosomes with $r \leq 2$
z: largest/smallest chromosome ratio.

II.4

CHARACTERIZACION OF THE PLASTID GENOME IN HEMIPARASITES

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ABSTRACT

The degree of sequence conservation of the plastid DNA (cpDNA) of three hemiparasites, *Thesium humile*, *Striga hermonthica* and *Striga gesnerioïdes* has been established. We found that *Thesium humile* and *Striga hermonthica* cpDNAs are about 130 kb and *Striga gesnerioïdes* cpDNA is about 115 kb as compared with tobacco cpDNA which is 155.5 kb. The most conserved regions within the cpDNA are the inverted repeat that contain genes for ribosomal RNAs. All of the photosynthetic genes studied are present in the hemiparasites, whereas the *rpo* genes coding for RNA polymerase subunits exhibit some differences with those of tobacco. Expression of the *rpo* genes has not been demonstrated. This would imply the necessity to import these subunits from the cytosol to express all the photosynthetic genes which have been maintained in the plastid genome. Moreover, *rpo*A has probably migrated to another region of the cpDNA.

each gene to be sought, two regions of well conserved sequences, at least in tobacco (Shinozaki *et al.*, 1986) and rice (Hiratsuka *et al.*, 1989) have been used as primers.

Plastid isolation, DNA purification, amplification, digestion and hybridization were performed as previously described (Delavault and Thalouarn, 1994).

Results and Discussion

Plastid DNA organization

The size of the plastid chromosome was estimated after digestion by *BamHI* restriction enzyme and electrophoresis (Fig. 1). At least 26 and 23 fragments were resolved in 0.8% agarose gel for *Thesium* and *Striga* respectively. From this it could be deduced that *Thesium* and *S. hermonthica* cpDNAs are about 130 kbp while *S. gesnerioïdes* cpDNA is about 115 kbp in size.

Using a clone bank of twelve restriction fragments covering the entire tobacco (N.t) plastid DNA, it can be concluded that no major change has occurred in the structure and organization of the chloroplast chromosome of the hemiparasites studied. They contain a 20-25 kbp inverted repeated sequence ; the repeats are separated by a small (3-9 kb) and a large (60-65 kb) single copy region. A comparison with tobacco shows that the LSC (90 kb in N.t) and especially the SSC (19 kb in N.t) have undergone significant reduction in size whereas the most conserved regions are those within the IR (25 kb in N.t).

In the LSC of *Striga* and *Thesium* the most deleted region hybridizes with the overlapping probes pTB20 and pTB7. In tobacco these fragments contain several genes : 3 *rpo* genes, 2 *psa*, 3 *psb* and several *trn* genes for transfer RNAs (Fig. 2).

It is not easy to estimate accurately the size of the SSC in cpDNA of hemiparasites since no hybridization signal is obtained with the 18.1 kb probe pTBa2 which corresponds almost entirely to the SSC in tobacco cpDNA (Fig. 2). Only one out of three *XhoI/PstI* subfragments of Ba2 hybridizes with *Thesium* cpDNA. No hybridization signal is detected when *Striga* cpDNA is hybridized with these probes. However, a 17 kb fragment that hybridizes with the Ba5 probe from tobacco IR represents, at least partially, the SSC since this region is surrounded with two Ba5 fragments of 7.1 kb each. Hence almost 3 kb correspond to *Striga hermonthica* SSC, this region being almost 4 kb in *S. gesnerioides*.

The inverted repeat in *Thesium* and *Striga* is nearly identical to that of tobacco (94% of its size) and it can be expected that their genic content is similar. From our results it can be concluded that the plastid DNA of the hemiparasites *Thesium* and *Striga* is far less reduced in size than those of holoparasites like *Epifagus, Orobanche hederae* or *Lathraea*. However, in both the hemi- and holoparasites the organization of the IR, LSC and SSC and the gene order on the chromosome are maintained in a manner similar to that found, in photosynthetic higher plants such as tobacco. But other characteristics of the cpDNA are only shared by parasitic plants, either hemi- or holoparasites : the deletions have occured mainly in the LSC and the SSC while the IR is well conserved. A similar result has been obtained by Haberhausen and Zetsche (1994) who demonstrated that all *ndh* genes (localized in the SSC in higher plants) are lost or significantly altered in the hemiparasite *Cuscuta reflexa*.

Gene content

Among the 12 genes chosen to illustrate the maintenance of the functionality of the plastid DNA in *Thesium* and *Striga*, 8 code for proteins involved in the photosynthetic process and 4 for the gene expression apparatus. When heterologous probes from tobacco were hybridized to the cpDNAs, hybridization signals were detected in most cases. However, when *rpl20* and *rpoA* probes from tobacco were hybridized to *S.*

hermonthica and *S. gesnerioides* cpDNA no signal could be detected suggesting deletion or important changes in the nucleotide sequence. PCR was used to demonstrate that the coding region of these genes has been wholly maintained. Amplification and control by Southern blotting led us to conclude that these genes have the same size as those of tobacco. However, to obtain an amplification of *rpoA* of *S. hermonthica* and *S. gesnerioides* or of *psaB* in *S. gesnerioides* and *atpB-E* in *Thesium* and *S. gesnerioides*, the annealing step of the PCR programme was conducted at 50ºC and 45ºC respectively instead of 55ºC. This suggests that gene nucleotide sequences which correspond to the primers are somewhat different from those of tobacco.

The most striking result was obtained with *rpoB* gene from *S. hermonthica* and *S. gesnerioides* which is impossible to amplify as a whole (3.2 kb) while amplified products of 1.6 or 1.4 kb result from this attempt. Nevertheless, analysis of the nucleotide sequence of the *Striga* 1.6 kb PCR product showed high homology with tobacco, especially in the 5' coding region. Very little is known about the *rpo* genes encoding for a plastid RNA polymerase in parasitic plants except that these genes do not exist in *Epifagus*, a finding which raised the possibility that gene transcription is not possible in *Epifagus* plastids. Yet, evidence of transcription of some plastid genes, such as 16SrRNA, was obtained by Northern hybridization (de Pamphilis and Palmer, 1990). In the case of *Striga*, further work is needed in order to test the following hypothesis : are *rpo* genes markers of a slight degradation in the plastid DNA of hemiparasites? However the functionality of this DNA in *Thesium* and *Striga*, evidenced by Northern hybridization (with *rbcL* transcripts) remains beyond doubt.

From this work, it could be concluded that in the hemiparasites studied here, the plastid genome, while significantly reduced in size, has been roughly maintained in organization, gene content and order. However some of the genes may have undergone changes in their length or nucleotide sequence and therefore deserve to be studied in further detail. This is certainly the case for the *rpoB* gene. Such a study might give rise to important findings related to the occurrence of the first effects of parasitism on cpDNA or identify a gene interesting in phylogenetic studies.

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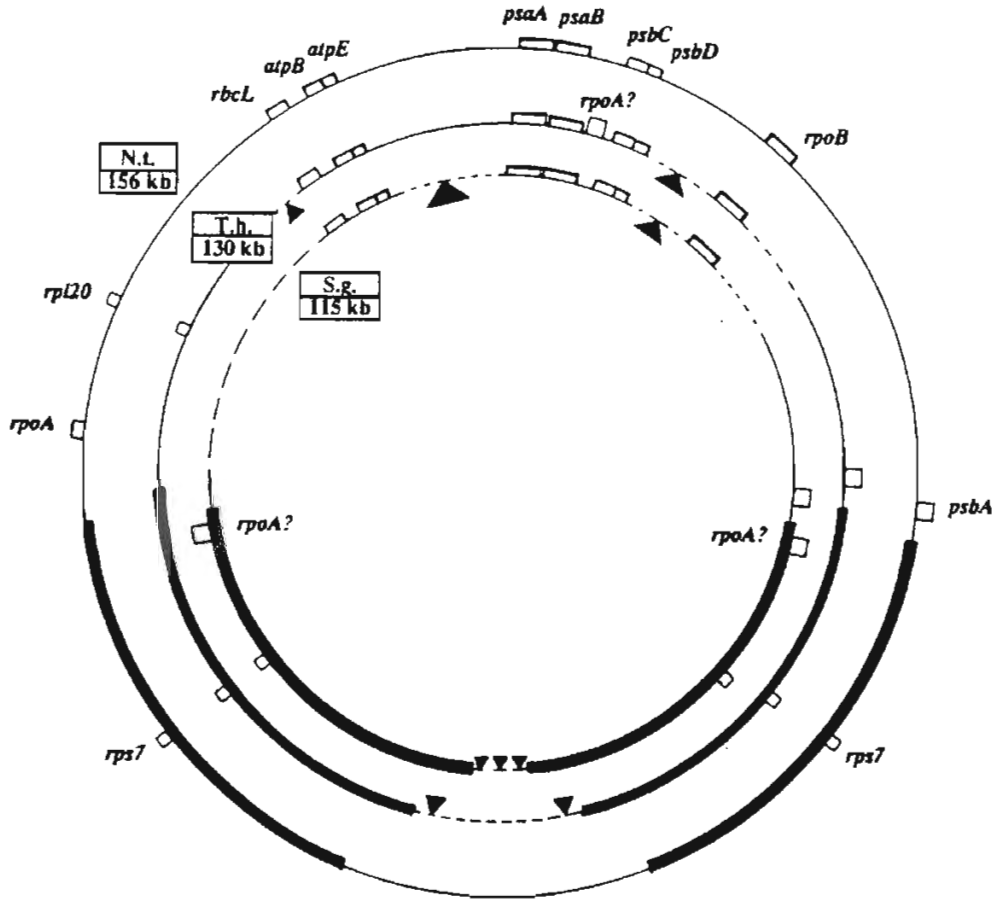

Figure 1

Plastid DNA organization and partial gene map of tobacco (N.t.), *Thesium humile* (T.h.) and *Striga gesnerioides* (S.g.). Genomes are in proportion to the circle sizes. IR segments are indicated with thickened lines. Black triangles represent the regions where large deletions have occurred. Regions deleted in a lesser extent are indicate in dashed lines.

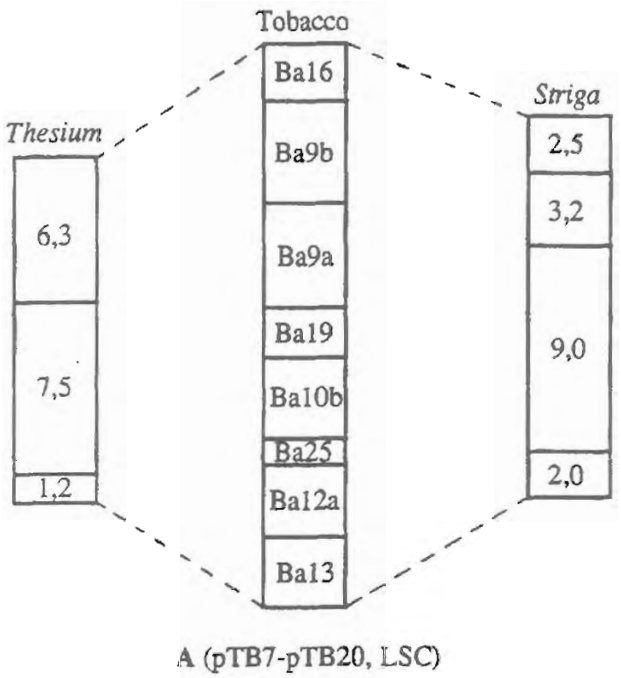

Figure 2

Representation of the main deleted regions in *Thesium* and *Striga* plastid genome.
A: in a part of the large single copy (LSC) that corresponds to pTB7 and pTB20 fragments in tobacco. Subfragments in pTB7 and pTB20 are numbered Ba16, Ba9b, etc...
B: in the small single copy (SSC). *BamHI*, *PstI* and *XhoI* are restriction sites on tobacco SSC. Numbers indicate size in kilobases.

II.5

AN HYPOTHETIC HISTORY OF *Striga* - A PRELIMINARY DRAFT

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ABSTRACT

Several species of *Striga* and one of *Buchnera* are crop parasites. We propose a comparison of these closely related genera, concerning : floral organization, parasitism and distribution. *Striga* can be considered as more advanced than *Buchnera* in specialization of floral biology as well as in parasitism. Because of their distributions, *Buchnera* may have originated around the end of Palæocene, while *Striga* probably arose much later, around the end of Eocene. The differentiation of *Striga* species occured probably in successive phases. In the evolutionary trend of annual species, the adaptation to crops is considered as the most advanced behaviour.

Additional key words : Scrophulariaceæ, evolution, speciation.

2. Hypothetical history of *Striga*

After having studied the geographical distribution of some striking characters observed in *Striga* species, and the rate of endemic species in each region where *Striga* is to be found (Raynal-Roques, 1994 a), we can propose, for the genus *Striga*, a schema of diversification in successive stages.

- 1st stage) The genus *Striga* may have originated in Africa, where we observe the greatest number of species and of endemic taxa, and the greatest bio-morphological diversity. A first speciation phase occured in Africa ; these early species were likely annual, hemiparasitic, leafy plants, the erect stem ending in a loose spike of zygomorphic flowers.

- 2nd stage) During a second phase, the genus spread to Asia, following the southern bank of Thethys Sea up to Sunda Islands, and finally reached Australia.

- 3rd stage) The genus has diversified in Africa, during a third phase. Endemic species arose, quite different from the early ones, being distinguished by striking characters as holoparasitic biology, perennial life-cycle, reduced leaves, low plants whose aërial stems are reduced to flowering spikes, strongly zygomorphic calyx ... The biological adaptations of these species (Raynal-Roques, 1993) may be a response to the increase of ecological stresses happening over the past very few millions of years on the continent.

- 4th stage) A fourth phase appeared recently, and is still going on. It is concerning annual species. Some of them are gradually shifting from native hosts in natural vegetation to crop-hosts in cultivated fields.

When expanding from the wild to cultivated fields, the plants follow a progressive sequence (Raynal-Roques, 1994 b) : coming from the savanna hosts, they first become occasional parasites on crops. Though noxious, their attacks are too rare to have any economic effect. They gradually become more adapted and destructive in crops, as shown in Table 2. Among the 8 species, some may be considered as just beginning their adaptation (*S. passargei* Engl. is common on native plants, but scarce on sorghum ; it is a little important parasitic weed). The only species of *Buchnera* known to parasitize crops (*B. hispida*, occasionally on millet and sorghum in West Africa) belongs to this category. Some other species of Table 2, as *S. aspera* (Willd.) Benth. for example, are frequent on crops. Fairly common in african savannas, this species has been known on sorghum for about 25 years. It has also been observed on sugarcane, millet, dry rice. Its evolution as a crop-parasite has been favoured by its adaptation to maize, which has been widely planted in the past 20 years. In this process, the *Striga*s become gradually more competitive in crops than in natural vegetation, becoming more widely distributed in fields than in the wild.

The evolutionary process reaches a high level of adaptation to anthropophilous ecology. This last step is reached by *S. hermonthica*. Throughout its wide geographical range, it is the most important pest in crops, but in native vegetation, it seems to be restricted to Ethiopian region (Table 2). It probably started crop-parasitizing at the time of the beginning of agriculture in tropical Africa, and expanded with it. As it was spreading, it became more and more adapted and noxious to crops. As far as we know *S. hermonthica* is the most crop-adapted species, we better understand how serious the agronomic problem is.

Conclusion

The genus *Striga* started its diversification during the last period of Tertiary. The present-day phase of its specialization is a gradual adaptation to crops in cultivated fields. *Striga hermonthica* has probably been the first species to shift from native vegetation to crops, as early as the beginning of agriculture. It

shows the closest adaptation to this man-modified ecology, and is also the most noxious weed. Some other species are less adapted to crops and less noxious. Their evolution in this way is likely more recent, and they are moving towards a weedy biology. As a consequence, the number of crop-parasites is probably going to increase while the agronomic importance of each of them will grow in importance.

The only crop-parasitic species of *Buchnera* is at an early stage of adaptation to cultivated fields. This genus, though older than *Striga*, is less specialized. It seems to have, up to now, less evolutionary potentialities .

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Table 1

COMPARISON *BUCHNERA-STRIGA*: DIFFERENTIAL CHARACTERS RELATED TO FLORAL BIOLOGY AND PARASITIC BIOLOGY (NEUMANN, 1995; OUÉDRAOGO, 1995; RAYNAL-ROQUES, 1994 A; SALLÉ & AL., 1995)

BUCHNERA	*STRIGA*
floral morphology	
corolla nearly actinomorphic	corolla strongly zygomorphic.
corolla tube nearly straight	corolla tube abruptly kneeled.
corolla limb nearly rotate	corolla limb two-lipped.
corolla lobes subequal	corolla lobes unequal, two of them comparatively small, more or less united (upper lip); the three others widely spreading (lower lip).
no mat of down-pointed straight hairs in the corolla tube	a mat of retrorse straight hairs under the attachment of the stamen filaments, in the corolla tube.
no club shaped hairs	club shaped hairs often present on the inner surface of the upper lip.
a ring of lomentaceous hairs at the corolla mouth	no ring of hairs at the mouth.
parasitic biology	
hemiparasites, but occasionnally able to carry out their biological cycle without a host	plants always connected to a host, hemiparasites or holoparasites (4 species holoparasitic).
the seed has no resting period	the seed needs a resting period before beeing able to germinate.
the seed needs no germination stimulant	germination depends on a chemical stimulant produced by a host root.
a single species *(B. hispida)* is, occasionnally and scarcely, a crop parasite	11 species have adapted to cultivated hosts and some of them are effective pests on food-crops.

Table 2

THE PROGRESSIVE ADAPTATION OF *STRIGA* SPECIES TO CULTIVATED FIELDS

SPECIFIC NAME	DISTRIBUTION IN NATURAL VEGETATION	DISTRIBUTION ON CROPS	MAIN CROP-HOST
1 — minor pests			
S. angustifolia	Eastern Africa, Asia, Indonesia	East Africa (Malawi and Tanzania), Asia (from India to Thailand)	Maize, millet, dry rice, sorghum, sugar-cane.
S. aspera	Africa	West Africa	*Digitaria exilis*, maize, millet, rice, sorghum, sugar-cane.
S.densiflora	Asia (mainly India)	India	Maize, millet, sorghum, sugar-cane.
S.forbesii	Africa, Madagascar	East Africa	Maize, dry rice, sorghum, sugar-cane.
S.latericea	East Africa	Ethiopia, Somalia	Sugar-cane.
S.multiflora	Australia	Australia	Sugar-cane.
S.parviflora	Australia	Australia	Sugar-cane.
S. passargei	Western- and Central-Africa	Burkina Faso and Mali	Sorghum.
2 — major pests			
S. asiatica	Africa, Madagascar, Asia.	Africa (mainly East-Africa), Madagascar, Asia; (U.S.A., not native).	Maize, millet, dry rice, sorghum, sugar-cane
S. gesnerioides	Africa, Cabo Verde Islands, western Asia	Africa, India ; (U.S.A., not native)	Cowpea, sweet potatoe, tobacco
3 — the most important pest			
S. hermonthica	Northern East Africa	Africa, Madagascar, western Asia	*Digitaria exilis, Eleusine coracana, Eragrostis abyssinica*, maize, millet, oats, dry rice, rye, sorghum sugar-cane

1 - Minor pests on crops : they are locally damageable, and widespread in native vegetation. Their distribution as crop-parasites is restricted to patches, enclosed in their distribution in the wild.

2 - Major pests : very damageable. Their distribution as crop-parasites is wider than their naturazl one.

3 - The most important pest on crops. It is widely distributed on crops but it is very localized in native vegetation.

II.6

AN INSIGHT INTO THE POPULATION STRUCTURE AND GENETIC DIVERSITY OF *Striga hermonthica* IN WEST AFRICA

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ABSTRACT

The population structure and genetic diversity of *Striga hermonthica* was studied using gel electrophoretic analysis of proteins from seeds of individual plants from 14 populations growing on sorghum, millet, maize and wild grasses in Burkina Faso, Mali and Niger. The genotypic constitution of each sample was depicted for two loci using the relative intensity of the different bands within a pattern. Genotypic frequencies conformed to Hardy-Weinberg expectations in 13 populations out of 14 for each locus. Heterozygote deficiencies could be the result of the Wahlund effect. Based on the loci that were interpreted, the genetic divergence between populations appears to be low. The physiological specialization for a particular host could then be a recent phenomenon. A low host-specificity of *S. hermonthica* populations could affect the efficiency of introducing new resistant cultivars as a control measure against the parasite.

Additional key words: witchweed, isozymes.

Results

Genotypic analysis of *Adh* and *Got* loci

Although no cross-breeding experiment was performed to document inheritance patterns, the interpretation of the zymograms appeared simple enough to allow the achievement of a genotypic-like analysis. With ADH, we effectively observed that *S. gesnerioides*, an autogamous species, invariably presented a single-band zymogram, which likely corresponds to one single allele at one single locus. On the other hand, *S. hermonthica*, which is an allogamous species (Safa et al., 1984), presented three or five-band patterns only. With a dimeric enzyme, a three-band pattern is expected to occur when two major alleles are present in a population, the central band representing the heterodimer and the two extreme ones representing two distinct homodimers. As the dimeric nature of ADH has been reported in numerous plants, we could then postulate that a dimer enzyme / one locus / two alleles hypothesis would likely explain the three-band pattern observed in *S. hermonthica*. The presence of a third allele in a few populations would explain the occurrence of some five-band patterns. The same reasoning was applied to GOT zymograms, whose dimeric nature has been demonstrated in numerous plants also.

We used the relative intensity of the different bands within a pattern (assessed visually and by means of a DESAGA CD 60 densitometer) to depict the genotypic constitution of each sample. This was possible because a theoretical intensity of band coloration can be calculated using the matrices of the relative allele frequencies of all possible genotypes and the enzyme contribution of each genotype. To do that, however, we had to assume that the relative intensity of band coloration is roughly proportional to enzyme concentration and that all alleles are evenly expressed, thus permitting to infer gene dosages.

To attribute a genotype to the mother plant, the different possibilities were tested with a range of hypotheses of allele frequencies for the pollen population. As only a few distinct types of zymograms were observed, this approach usually allowed only one genotype to be compatible with the banding pattern observed. The allele frequencies in the pollen population were then roughly estimated. The allele frequencies were also calculated based on the population of mother plants, and compared with those of the pollen population. Lastly, the panmictic distribution was tested based on the population of mother plants. Observed genotype frequencies were compared to those expected under Hardy-Weinberg equilibrium using a Chi-square test for goodness of fit (Li and Horvitz, 1953). When more than two alleles occurred at a locus, a pooling of the genotypes into three classes was performed: homozygotes for the most common allele, heterozygotes for the most common allele and one other allele, and all other genotypes. Wright's (1978) fixation index (F) was used to determine heterozygote deficiencies or excesses.

Allele frequencies of *S. hermonthica* populations

Calculated allele frequencies for the *Adh* and *Got* loci are presented in Table 1 for all populations and for six subpopulations from mixed crops. Observed and expected frequencies of heterozygotes, and Wright's fixation index, are summarized in Table 2. All the populations studied were in Hardy-Weinberg equilibrium for both loci, except population 3 for the *Got* locus, and population 11 for the *Adh* locus, where heterozygote deficiencies were observed.

Discussion

Much of the information drawn in this study relies on the genetic interpretation of ADH and GOT variation. Among various possible genetic models, we chose the one locus model. The occurrence of ADH gene duplication that results from either polyploidy or chromosomal rearrangements has

been reported in a few plant species (Gottlieb, 1981; Ellstrand et al., 1983). It is unlikely, however, that this rare event would apply to *Striga* species. *S. gesnerioides*, for example, always shows one-band patterns. It is unlikely that a duplicate gene, e.g. two loci, have the same fixed allele. All the results obtained with *S. hermonthica* are also coherent with the simpler single gene model. We also used simple rules for translating the banding intensities in terms of bulk genotypic constitution. These rules may have some exceptions, but they appear relevant in all the abundant literature on isozymes. Similarly, we omitted to consider possible sources of deviations, such as gametic selection, in calculating theoretical genotypic frequencies. All the results, however, form a scheme which is likely to be valid, given its global coherence.

In this report, the two loci analysed are in Hardy-Weinberg equilibrium for most populations. This is consistent with the notion of an outcrossed species where the populations are panmictic through random pollinization and fecundation. The zymograms observed within each population were also consistent with allogamy and panmixia for both *Adh* and *Got* loci. With most of the zymograms, there was a concordance between the pollen contribution to the mother plant that would explain the banding pattern observed with the progeny, and the calculated allele frequencies in the population. Thus, it is likely that the mating events happen randomly with a trend towards the panmictic equilibrium.

Although heterozygote deficiencies were observed, using Wright's fixation index, this occurred with only one population out of 14 for both *Adh* and *Got* loci. Such rare events could have happened at random. They could also be the result of non-random pollinization, as suggested by Bharathalakshmi et al. (1990). Most likely, the cause of this would be the Wahlund effect, e.g. the population studied would be divided into subpopulations slightly different for their allele frequencies, and inside which the pollinization would occur randomly. It is possible that a few plants in a population escape from panmixia because the pollinic near-environment is distinct and the pollinization involves more pollen from the nearest neighbors than from the rest of the population. Effectively, the pattern of distribution of *S. hermonthica* in the field is rarely uniform. Thus, the possibility that some populations are divided into subpopulations cannot be completely rejected. However, the low occurrence of heterozygote deficiencies through the populations studied would be in agreement with the hypothesis that *S. hermonthica* is a highly allogamic species which is under panmixia.

The results of our study seem to indicate that the allelic divergence among populations of *S. hermonthica* is low for *Adh* and *Got* loci. All the genetic variability observed at these two loci was generally present in every population, with the exception of a few rare alleles. The interpopulation diversity appears to stand only on differences in the frequencies of the main alleles, and on the introduction of new rare alleles. Although it would be hazardous to extrapolate from these results as they concern a very low number of loci, the trend is consistent with what we could expect from an outcrossed species like *S. hermonthica*, which is widespread throughout Africa. This is also in agreement with the study of Bharathalakshmi et al. (1990), which revealed high values of identity between three *S. hermonthica* populations.

The investigation of the results also indicates that there does not seem to be any geographical cline among Burkina Faso, Mali and Niger populations. Furthermore, no obvious correlation could be established between allele frequencies and the host plant species. The analyses of populations of *S. hermonthica* growing on mixed crops provided similar information, as subpopulations did not present any difference related to the host. This suggests that the specialization of *S. hermonthica* for its host is likely to be a recent phenomenon, although the hypothesis of fixed specificity cannot be rejected because the loci studied may not be representative of most other loci. It is possible that every *S. hermonthica* population, because of its

Table 1

ALLELE FREQUENCIES FOR THE *ADH* AND *GOT* LOCI IN 14 POPULATIONS AND 6 SUBPOPULATIONS OF *S. HERMONTHICA*

LOCUS	POPULATION														SUBPOPULATION					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	4S*	4Ma*	9S	9Mi*	12S	12Ma
Adh																				
(N)	20	30	47	22	28	17	57	18	45	22	19	35	24	23	13	9	35	10	24	11
A	0.45	0.53	0.51	0.77	0.50	0.35	0.63	0.53	0.57	0.41	0.50	0.40	0.44	0.59	0.77	0.78	0.59	0.55	0.44	0.32
B	0.53	0.48	0.49	0.23	0.48	0.62	0.32	0.47	0.37	0.57	0.50	0.60	0.56	0.39	0.23	0.22	0.36	0.35	0.56	0.68
C	0.03	0.00	0.00	0.00	0.02	0.00	0.03	0.00	0.07	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.06	0.10	0.00	0.00
D	0.00	0.00	0.00	0.00	0.00	0.03	0.03	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Got																				
(N)	20	17	36	-	14	9	39	16	35	10	16	13	9	17	-	-	29	6	8	5
A	0.93	0.97	0.53	-	0.64	0.56	0.77	0.84	0.91	0.75	0.78	0.89	0.72	0.71	-	-	0.91	0.92	0.94	0.80
B	0.08	0.03	0.47	-	0.36	0.44	0.23	0.16	0.09	0.25	0.22	0.12	0.28	0.29	-	-	0.09	0.08	0.06	0.20

* S = sorghum, Ma = maize, Mi = millet population.

Table 1

OBSERVED AND EXPECTED NUMBERS OF HETEROZYGOTES, WRIGHT'S FIXATION INDEX (*F*) AND SIGNIFICANCE OF THE CHI-SQUARE TEST FOR PANMIXIA IN 14 POPULATIONS AND 6 SUBPOPULATIONS OF *S. HERMONTHICA* FOR THE *ADH* AND *GOT* LOCI

	ADH					*GOT*				
Pop.	N	Obs. heteroz.	Expect heteroz.	F	Chi2	N	Obs. heteroz.	Expect heteroz.	F	Chi2
1	20	9	9.975	0.098	N.S.	20	3	2.775	-0.081	N.S.
2	20	7	9.975	0.298	N.S.	17	1	0.971	-0.030	N.S.
3	47	28	23.489	-0.192	N.S.	36	10	17.944	0.443	**
4	22	8	7.727	-0.035	N.S.	-	-	-	-	-
5	28	12	14.000	0.142	N.S.	14	6	6.429	0.067	N.S.
6	17	9	8.029	-0.121	N.S.	9	4	4.444	0.100	N.S.
7	57	30	26.526	-0.131	N.S.	39	14	13.846	-0.001	N.S.
8	18	9	8.972	-0.003	N.S.	16	3	4.219	0.289	N.S.
9	45	23	22.100	-0.041	N.S.	35	6	5.486	-0.094	N.S.
10	22	9	10.795	0.166	N.S.	10	3	3.750	0.200	N.S.
11	19	5	9.500	0.474	*	16	7	5.489	-0.280	N.S.
12	35	22	16.800	-0.310	N.S.	13	3	2.654	-0.130	N.S.
13	24	15	11.813	-0.270	N.S.	9	3	3.611	0.169	N.S.
14	23	11	11.152	0.014	N.S.	17	6	7.059	0.150	N.S.
4S	13	6	4.615	-0.300	N.S.	-	-	-	-	-
4Ma	9	2	3.111	0.357	N.S.	-	-	-	-	-
9S	35	17	16.986	-0.001	N.S.	29	5	4.569	-0.094	N.S.
9Mi	10	5	4.950	-0.010	N.S.	6	1	0.917	-0.091	N.S.
12S	24	15	11.813	-0.270	N.S.	8	1	0.938	-0.067	N.S.
12Ma	11	7	4.773	-0.467	N.S.	5	2	1.600	-0.250	N.S.

* Significant ($P < 0.05$)

** Significant ($P < 0.01$)

N.S. Non significant

Introduction

Striga hermonthica (Del.) Benth. is one of the economically most important species of the parasitic weed *Striga*, causing losses in the yields of a number of crops in sub-sahelian countries in Africa. Although it mainly occurs on crops and grasses nearby arable fields and seems to be completely adapted to the agroecosystem, it is occasionally found in the natural vegetation, far from agricultural land, in Sudan and Ethiopia (Musselman *et al.*, 1991; Reda, personal communication). *Striga aspera* (Willd.) Benth. is considered a minor parasitic weed, mainly occurring on wild grasses. In the last 10 years it has been found more and more often on maize and appears to be a potential threat to crops (Raynal-Roques, 1994).

The species are closely related, both in their physical appearance as well as in their biology. While allogamy is the usual mode in *Striga*, these are the only two species which are obligate outcrossers (Musselman *et al.*, 1991). It is (often) difficult to distinguish between them in areas were they overlap in West Africa. Usually the bend in the corolla tube is used as a character for distinction between the species (Parker and Riches, 1994). *Striga hermonthica* flowers have the bend almost exactly half way while in *S. aspera* flowers it is at least two-thirds up the tube. This character is reliable to distinguish both species in West Africa, but in East Africa, especially Kenya, there is a lot of variation in the location of the bend in the corolla tube of *S. hermonthica* flowers (personal observation). In some fields most plants had flowers with a bend distinctly above half-way (Kenyan form), making it closer to the *S. aspera* pattern. Plants with this morphology are also found in Uganda and Tanzania (Parker, 1991). However, in Kenya there were also fields were plants had flowers which resembled more the West African pattern.

Because the distinction between the two species on morphological criteria is biased at least in some areas, it would be interesting and worthwhile to evaluate a possible genetic differentiation based on molecular genetic markers (allozymic). The main objective of this study is therefore to determine differences in genetic diversity between *S. hermonthica* and *S. aspera* from West and East Africa. In addition it could reveal whether the difference in flower morphology of Kenyan *S. hermonthica* populations is due to a genetic differentiation.

The results of the allozyme screening are discussed in view of additional crossing studies and the potential impact this species complex could have on crop species.

Materials and Methods

Striga populations and number of plants used

Seeds of *S. aspera* populations were collected in West Africa. Seeds of *S. hermonthica* populations were collected in both West and East Africa (Kenya). Kenyan *S. hermonthica* populations were subdivided on the basis of their flower morphology (bend in the corolla tube). Populations from cereal hosts were collected in the fields, populations from wild hosts were collected far away from agricultural fields, unless specified otherwise. Populations came from the following sites (numbers correspond to those in the text):

1 *S. aspera* from maize, Garoua in Cameroon, 1989.
2 *S. aspera* from maize, Dedougou in Burkina Faso, 1994.
3 *S. aspera* from upland rice, Kouto in Ivory Coast, 1992.
4 *S. aspera* from wild grasses, Sotuba in Mali, 1994.
5 *S. aspera* from wild grasses, Cinzana field station in Mali, 1994.
6 *S. hermonthica* from maize, Zakpota in Benin, 1993.
7 *S. hermonthica* from wild grasses near a maize field, Rice Station near Kana in Benin, 1993.

8 *S. hermonthica* from sorghum, Tiefala in Mali, 1984.
9 *S. hermonthica* from maize, Leo in Burkina Faso, 1994.
10 *S. hermonthica* from pearl millet, Dakiri in Burkina Faso, 1994.
11 *S. hermonthica* from maize, Rice Station near Kana in Benin, 1993.
12 *S. hermonthica* (Kenyan form) from wild sorghum/*Rottboellia* spp., at the entrance of the Ruma National Park in Kenya, 1993.
13 *S. hermonthica* (Kenyan form) from maize, Kibos in Kenya, 1993.
14 *S. hermonthica* (Kenyan form) from sorghum, Alupe in Kenya, 1993.
15 *S. hermonthica* (Kenyan form) from wild grasses just outside a maize field, Kibos in Kenya, 1993.
16 *S. hermonthica* (Kenyan form) from *Bottriochloa insculpta*, inside Ruma National Park in Kenya, 1993.
17 *S. hermonthica* (West African form) from wild sorghum, Kibos in Kenya, 1993.
18 *S. hermonthica* (West African form) from sorghum, Bumala in Kenya, 1993.
19 *S. hermonthica* (West African form) from wild sorghum/*Rottboellia* spp., at the entrance of the Ruma National Park in Kenya, 1993.
20 *S. hermonthica* (West African form) from maize, Kibos in Kenya, 1993.

Thirtysix plants of each population (grown from seeds in *in-vitro* cultures) were screened for their allozyme variation. Due to the fact that no more than 10 samples could be analysed in one run, it was decided to use a reference population to correct for differences between runs of one population. For this purpose a *S. asiatica* population (from wild grasses near Homa Bay in Kenya; collected in 1993) was used. Initial studies with *S. asiatica* indicated that *S. asiatica* populations were almost completely homozygous for all the enzyme systems tested, and that banding patterns were completely different from those of *S. hermonthica* and *S. aspera*. The banding pattern of the reference in separate runs was used for recalculating differences in migration distances of the enzymes. Also several control runs were performed. In these runs samples (already tested) from nine different populations, and a reference, were compared to check allele designations.

In-vitro culture and harvest of *Striga* plants

Striga seeds were surface sterilised in 70% (v/v) ethanol for 5 min, followed by 10 min in 100% (v/v) commercial bleach. After thorough rinsing with deionized water, seeds were transferred to sterile glass containers (85 mm diameter, 380 ml) filled with 90-100 ml agar growth medium. The growth medium was modified after Cai *et al.* (1993). It's composition (1 litre) was: sucrose (20 g), bactoagar (10 g), MS-salts (4.5 g), myoinositol (0.1 g), ascorbic acid (10 mg), biotin (0.5 mg), indole-3-acetic acid (1.0 mg) and kinetin (0.5 mg). Containers were placed in a growth cabinet with a 12 h day/night cycle (day temperature 30 ± 0.1C; night temperature 27 ± 0.1C; light intensity at the level of the containers was 200-250 $mol.s^{-2}.s^{-1}$. To increase germination levels, 3 ml of a 10 ppm GR-24 solution was added to each container after 7 days.

Striga seedlings (whole plants) were harvested after approximately 40 days of growth. At least 36 individual plants of each population were frozen in liquid nitrogen and lyophilised. Dry plant material was stored in a vacuum exsicator. No significant losses of enzyme activity were detected for plant material stored in this way for about one year.

Sample Preparation

For each run 9 individual plant samples and one reference sample were used. 10-20 mg of plant material from each sample was ground with fine quartz sand in cold 0.1 M K-phosphate buffer (pH 7.3) containing 10 mM dithiotreitol (DTT), 0.15 % bovine serum albumin (BSA), 1.0 mM phenylmethane-sulfonylfluoride (PMSF), 1.0 % acetone and ± 0.25 g insoluble polyvinylpyrrolidone (PVPP, polyclar-AT).

The homogenates were centrifuged at 15000 rpm in a Kontron T-124 centrifuge (30 min at 4C). 0.8 ml of the supernatant of each sample was collected and filtered through Costar Spin-x 0.22 m micro centrifugal filters in an eppendorf centrifuge (5 min at 4C). Subsequently 400 l filtrate of each sample was transferred to microtiter wells and 5 l bromophenol blue was added as an indicator of the front during the allozyme run.

Electrophoresis

25 l of each sample was loaded onto a 6% acrylamide gel (Vertical PAGE; Desaga, Heidelberg) as described by Verkleij *et al.* (1986). Buffer systems used were: Gel buffer: 2.0 M Tris (pH 9.1); electrode buffer: 5.0 mM Tris, 38 mM glycine (pH 8.4 ± 0.1). Runs were performed at 50 mA constant current. Previous to each electrophoresis a pre-run was done for 20 min at 50 mA. The electrophoresis was terminated 45 min after the front had migrated out of the gels (total run time approximately 5 h and 30 min).

Enzyme staining

Eight enzyme systems were tested for their variation. These were (with enzyme commission number and staining procedure references in parentheses): Esterase (EST; E.C. 3.1.1.-; Soltis *et al.*, 1983), Glutamate dehydrogenase (GDH; E.C. 1.4.1.2; Soltis *et al.*, 1983), Leucine aminopeptidase (LAP; E.C. 3.4.11.1; Shaw and Prasad, 1970), Menadione reductase (MDR; E.C. 1.6.99.2; Shaw *et al.*, 1988), Malic enzyme (ME; E.C. 1.1.1.39; Siciliano and Shaw, 1976), Phosphoglucoisomerase (PGI; E.C. 5.3.1.9; Shaw and Prasad, 1970), Phosphoglucomutase (PGM; E.C. 2.7.5.1; Vallejos, 1983) and Shikimic dehydrogenase (SDH; E.C. 1.1.1.25; Vallejos, 1983).

Staining protocol for LAP (usually run under neutral pH conditions) was modified by increasing the strength of the Tris-maleate buffer to 0.6 M at pH 5.4. At lower concentrations Fast Black K salt precipitated from the solution shortly after a gel was placed in the staining buffer and no staining was observed.

Data Analysis

Genotypic loci were inferred directly from electrophoretic phenotypes and must therefore be considered putative since no crosses and genetic analysis were used to establish inheritance patterns.

Parameters for intrapopulation variability and interpopulation divergence were calculated from observed genotypic frequencies with the computer program BIOSYS-1 (Swofford and Selander, 1981). Due to the overwhelming amount of data obtained when analysing 20 populations, only banding patterns, F-statistics (Nei, 1977) and the cluster analysis (using the UPGMA algorithm (Sneath and Sokal, 1973) with Nei's (Nei, 1978) unbiased genetic identity) will be discussed in detail.

Results

Migration distances of all alleles of the eight enzyme systems are shown. In figure 1 we found a total of 14 loci for 8 enzymes. PGI showed four different loci (assuming it is a monomeric enzyme), EST, LAP and PGM two, and GDH, MDR, ME and SDH one. Mean number of alleles per locus was 2.8 in *S. aspera*, 2.4 in *S. hermonthica* from West Africa and 2.3 in *S. hermonthica* from Kenya. Observed heterozygosity values ranged from 0.091 to 0.239 and did not differ between *S. aspera* (mean Ho=0.170) and *S. hermonthica* (mean Ho=0.180). In several population significant deficits of heterozygotes were found for EST, GDH, LAP MDR and PGM.

A remarkable fact is that *S. hermonthica* and *S. aspera* share nearly all allozymic loci. There are only two species-specific loci; *Pgm-1* and *Lap-1* were present only in *S. aspera*.

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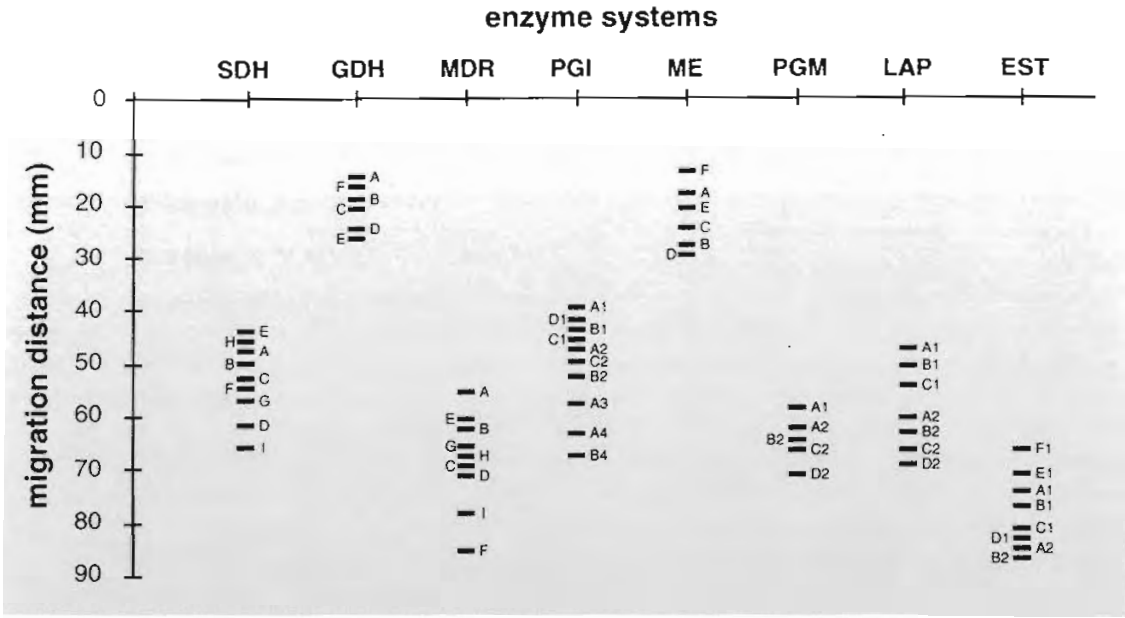

Figure 1

Migration distances of all alleles relative to each other for each of the eight enzyme systems tested. Shown here are their usual positions on the gels, but a 5 mm range should be taken into account on both sides, depending on differences in the gels. Alleles were numbered during the experiment, with new alleles given a higher alphabetic character.

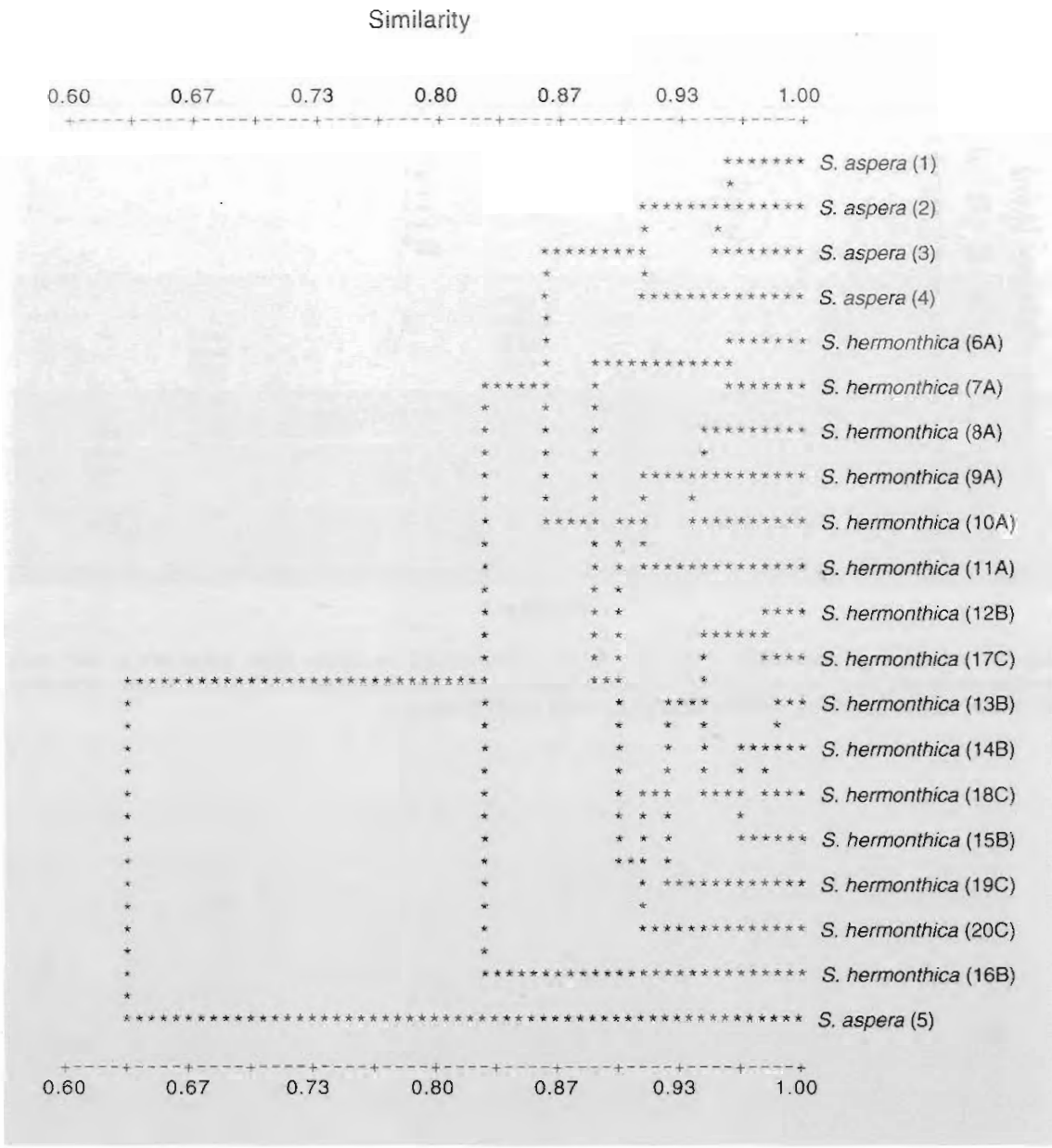

Figure 2

Hierarchical cluster analysis of the allozyme data using the unweighted pair-group method with arithmetic averaging (UPGMA) with Nei's unbiased genetic identity. Population numbers and flower form of *S. hermonthica* are between parentheses; A: *S. hermonthica* from West Africa, B: *S. hermonthica* from Kenya with the Kenyan flower form, C: *S. hermonthica* from Kenya with the West African flower form.

II.8

A Cladistic Investigation and Key to the Species of the Genus *Orobanche* L. Section *Trionychon* Wallr

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ABSTRACT

The taxonomy, biodiversity and status of *Orobanche* section *Trionychon* is investigated. Twelve species are recognized for section *Trionychon* and a key is provided. The evolutionary relationships of the species are investigated by cladistic methods. Species of section *Trionychon* are resolved as monophyletic with *O. caesia* and *O. lavandulacea* resolved at the base of the phylogenetic tree. The retaining of taxa of section *Trionychon* on each clade more or less correlates with the concept of crop parasitism and the range of biogeographical distribution.

Introduction

Orobanche is a genus of holoparasitic plants that includes several important agricultural pests. As noted by Musselman (1994), reliable taxonomy within *Orobanche* is desirable for improved understanding and access to the literature, especially for scientists concerned with control measures. However, this genus remains taxonomically difficult and imperfectly understood (Hepper, 1973; Musselman, 1986), partly because of the large number of characters with overlapping variation (Rumsey and Jury, 1991). These problems apply in particular to taxa of section *Trionychon*, including the *ramosa-aegyptiaca* complex and the unbranched broomrapes such as *O. arenaria* Borkh., *O. caesia* Reichenb. and *O. purpurea* Jacq. The large number of taxa previously ascribed to this section have recently been reduced to twelve species (Abu Sbaih, 1995). In this study, the relationships among these taxa is evaluated by cladistic analysis of morphological data. The main objectives of this analysis are to clarify the taxonomic status of section *Trionychon* within *Orobanche* and to determine relationships among its constituent species in relation to their biogeographical implications.

Materials and Methods

In addition to the twelve species belonging to section *Trionychon*, four species from section *Orobanche* and *Cistanche phelypaea* (L.) Coutinho are included in the analysis. Each species was scored for twenty characters as listed in Table 1. The data matrix was analyzed using the Branch and Bound search Algorithm of PAUP (Phylogenetic Analysis Using Parsimony) version 3.1.1 (Swofford, 1991). In initial searches, 700 equally parsimonious trees were found. Characters were reweighted by their rescaled consistency indices on these initial trees (Farris 1969, 1989) and the Branch and Bound search was repeated. This iterative reweighting process was continued the list of most parsimonious trees did not differ between iterations.

Results and Discussion

After four rounds of character reweighting a set of six most parsimonious trees was retained (L= 30214, CI= 0.765 and RI= 0.835). The 50% majority-rule consensus of these is shown in Figure 1. Synapomorphies of the genus *Orobanche* are the presence of glandular hairs on the stem, irregular flowers, corolla length and the presence of glabrous filaments. Section *Trionychon* is resolved as a monophyletic group defined by the presence of both tricin and and absence of luteolin aglycones (Abu Sbaih, 1995), presence of tricolpate pollen, pollen surface sculpturing, presence of bracteoles, shape of the calyx teeth, corolla colour and seed coat ornamentation (Fig. 1). Section *Orobanche* also is resolved as a monophyletic group by the absence of bracteoles, anther villosity, calyx tube and teeth shape, pollen type, corolla colour, corolla divergence and seed coat stratification. Although the two sections appear as sister taxa in our analysis, failure to include representatives of the other sections in *Orobanche* means that other relationships cannot be ruled out. Within *Trionychon*, *O. caesia* and *O. lavandulacea* Reichenb. form a sister group united by the presence of more inclined corollas and more or less rounded-ovate lower corolla lobes, which is sister to the reminder of the section. The remaining species form a clade on the basis of less inclined corollas and the degree of stamen insertion. *Orobanche purpurea* is resolved as an early divergence from these taxa. The remaining species form two clades according to anther villosity and the shape of the lower corolla lobes. The branched members of section *Trionychon* [*O. ramosa* L., *O. aegyptiaca* Pers., *O. oxyloba* (Reut.) G. Beck and *O. schultzii* Mutel] represent one clade united by the possession of branched stems and a microreticulate pollen surface sculpturing. Although *O. aegyptiaca* and *O. schultzii* are placed unambiguously with *O. ramosa-O. oxyloba* clade, relationships within this group cannot be furtherly resolved with the present data. *Orobanche nana* Noë, *O. rechingeri* Gilli and *O. bungeana* G. Beck form a monophyletic group defined by stem

length, bract length, calyx teeth shape and the sculpturing of the pollen surface. *Orobanche trichocalyx* (Webb and Berth.) G. Beck and *O. arenaria* are grouped together by having a similar seed coat ornamentation and stem branching pattern and length.

It is of considerable interest that the biogeography appears to be strongly related to phylogeny, such that the branched species which are predominantly wide-spread crop parasites (*O. ramosa* and *O. aegyptiaca*) form one monophyletic group, while the non-crop parasites (e.g *O. rechingeri*, *O. bungeana* and *O. nana*) which have a rather limited distribution in S. Europe and SW Asia form another.

It is evident that intensive studies of relationships both at the infraspecific level and at the higher supragenerіc levels within the family Orobanchaceae are required. Application of molecular techniques, particularly DNA sequence-based approaches, would be highly appropriate in this family where many potentially useful morphological and anatomical characters are unavailable because of reduction. Apart from the improved concepts of taxon delimitation that such studies could provide, the development of phylogenetic hypotheses would make a valuable contribution towards a truly predictive classification of the Orobanchaceae and potentially shed much light on the evolution of parasitic plants.

Key to Species of Section *Trionychon*

1 Anthers glabrous
2 Stem branched
3 Calyx teeth equalling tube or slightly longer; lobes of the lower corolla lip ± rounded-ovate; branches up to 10, usually basal ..... **1. ramosa**
3 Calyx teeth slightly shorter than tube; lobes of the lower corolla lip acute acuminate; branches 1-4 arising mainly on the middle to the upper part of the stem ..... **2. oxyloba**
2 Stem simple
4 Corolla tube less than 15 mm; calyx teeth filiform; stem 15x0.3 cm or less. ..... **5. nana**
4 Corolla tube 15 mm or more; calyx teeth acute-acuminate; stem more than 15x0.3 cm
5 Inflorescence woolly with white hairs; corolla tube nearly patent, inclined towards lower corolla lip 15-20 mm; bract broadly ovate. ..... **6. caesia**
5 Inflorescence glandular-pubescent, but not woolly with white hairs; corolla tube erecto-patent 15-25 mm; bract ovate to lanceolate
6 Calyx teeth longer than tube; corolla tube 20 mm or more, infundibular-campanulate, slightly dilated above, lobes of the lower corolla lip ± ovate ..... **7. purpurea**
6 Calyx teeth shorter than tube; corolla tube less than 20 mm, narrow infundibular, not dilated above, lobes of the lower corolla lip acute to acuminate ..... **2. oxyloba**
1 Anthers densely hairy
7 Stem branched
8 Corolla tube more than 20 mm ..... **3. aegyptiaca**
8 Corolla tube 20 mm or less
9 Calyx teeth ± equalling tube; corolla blackish-violet upon drying ..... **11. lavandulacea**

9 Calyx teeth distinctly longer than tube; corolla not blackish, but mostly yellow upon drying ..... **4. schultzii**
7 Stem simple
10 Plant 15 cm or less, inflorescence whitish, villous; corolla mostly pale yellow at base, not brownish when dried. ..... **8. rechingeri**
10 Plant more than 15 cm; inflorescence pale bluish- violet, villous; corolla bluish-violet, brownish when dried
11 Corolla tube exceeding 22 mm
12 Bracts broadly ovate 7 mm or less; bracteoles less than 7 mm; calyx teeth distinctly shorter than tube; corolla tube narrow infundibular, less dilated above ..... **9. bungeana**
12 Bracts ovate-lanceolate more than 7 mm; bracteoles more than 7 mm; calyx teeth equalling tube or longer; corolla tube divergent-infundibular, erecto-patent, dilated above ..... **10. arenaria**
11 Corolla tube less than 22 mm
13 Corolla tube strongly curved forward, blackish when dried; calyx teeth ± equalling tube ..... **11. lavandulacea**
13 Corolla tube not strongly curved forward, colour not blackish when dried; calyx teeth distinctly longer than tube
14 Stem up to 20 cm; corolla tube less than 17 mm, lower lobes ± acute-acuminate ..... **4. schultzii**
14 Stem above 20 cm; corolla tube 17 mm, or more, lower lobes ± rounded-ovate ..... **12. trichocalyx**

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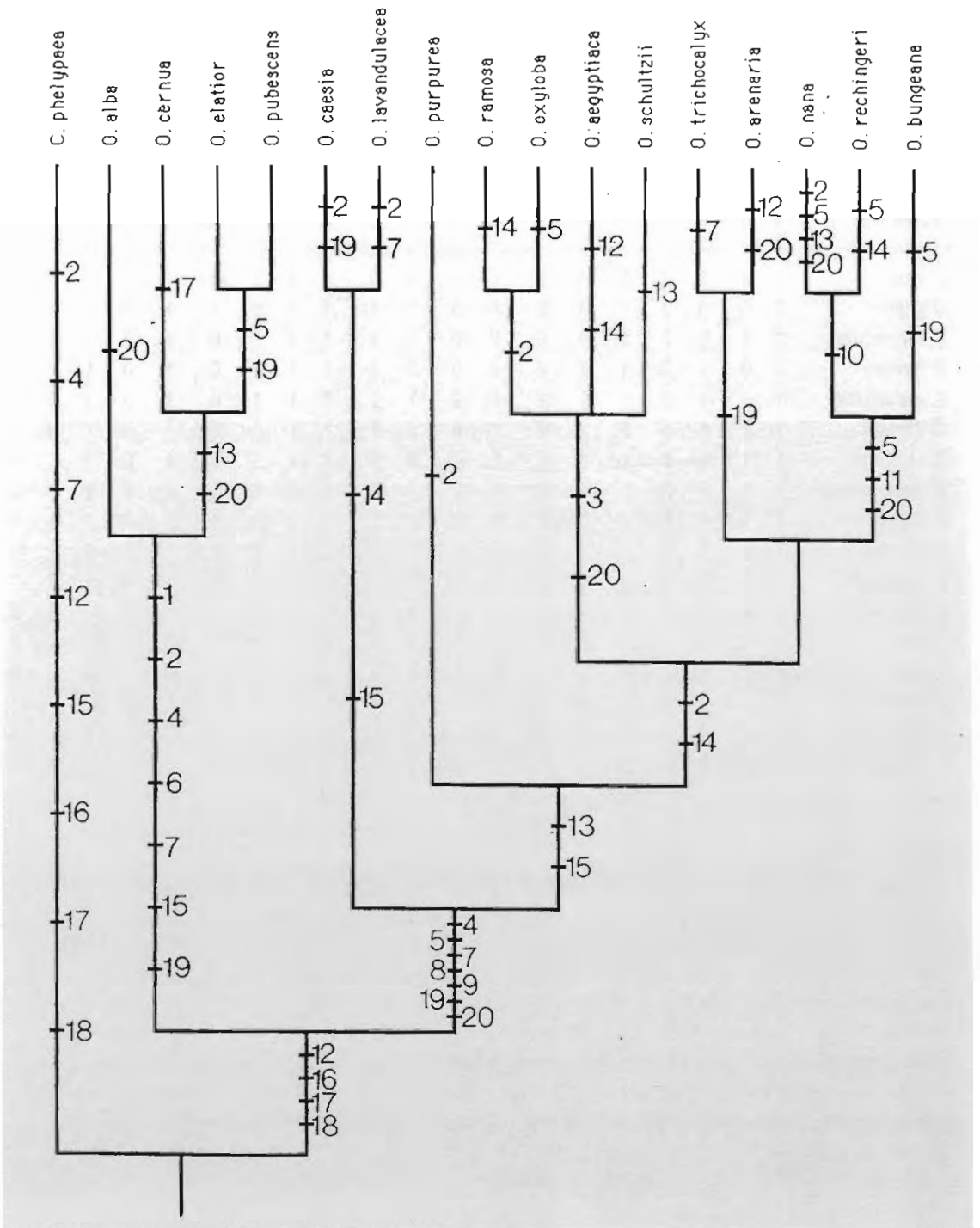

Figure 1

Majority rule consensus of six most parsimonious trees found from iterative reweighting search procedure. Numbers with bars indicates character changing along each branch.

Table 1

CHARACTERS AND CHARACTER STATES MATRIX OF *OROBANCHE* SPECIES AND *CISTANCHE PHELYPAEA*. *= MISSING VALUE

	CHARACTERS																		
										1									2
Species	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0
O. nana	1	0	0	2	0	1	2	1	0	0	0	1	0	2	0	1	0	1	0	1
O. caesia	1	0	0	2	1	1	2	1	0	1	1	1	0	0	1	1	0	1	1	2
O. trichocalyx	1	1	0	2	1	1	3	1	0	1	1	1	1	2	0	1	0	1	1	2
O. ramosa	1	0	1	2	1	1	2	1	0	1	1	1	1	0	0	1	0	1	0	0
O. aegyptiaca	1	1	1	2	1	1	2	1	0	1	1	2	1	1	0	1	0	1	0	0
O. oxyloba	1	0	1	2	0	1	2	1	0	1	1	1	1	2	0	1	0	1	0	0
O. rechingeri	1	1	0	2	1	1	2	1	0	0	0	1	1	1	0	1	0	1	*	*
O. lavandulacea	1	1	0	2	1	1	3	1	0	1	1	1	0	0	1	1	0	1	0	2
O. schultzii	1	1	1	2	1	1	2	1	0	1	1	1	0	2	0	1	0	1	0	0
O. arenaria	1	1	0	2	1	1	2	1	0	1	1	2	1	2	0	1	0	1	1	3
O. bungeana	1	1	0	2	0	1	2	1	0	1	0	1	1	2	0	1	0	1	3	*
O. purpurea	1	0	0	2	1	1	2	1	0	1	1	1	1	1	0	1	0	1	0	2
O. alba	0	0	0	1	0	0	1	0	1	1	1	1	0	1	1	1	0	1	2	4
O. cernua	0	0	0	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1	2	5
O. elatior	0	0	0	1	1	0	1	0	1	1	1	1	1	1	1	1	0	1	1	*
O. pubescens	0	0	0	1	1	0	1	0	1	1	1	1	1	1	1	1	0	1	1	5
C. phelypaea	1	1	0	0	0	1	0	0	1	1	1	2	0	1	0	0	1	0	*	*

Key to characters and their states.

1= bracteole presence or absence; 1= present, 0= absent. **2**= anther villosity; 0= glabrous, 1= hairy. **3**= stem branching; 0= simple, 1= branched. **4**= calyx teeth with a tube; 0= no tube, 1= half campanulate, 2= tube campanulate. **5**= calyx teeth shape; 0= shorter than tube, 1=longer than tube. **6**= pollen type; 0= inaperturate, 1= tricolpate. **7**= corolla colour; 0= yellowish, 1= not blue-violet 2= blue-violet 3= blackish violet. **8**= tricin presence or absence; 0= no, 1= yes. **9**= luteolin presence or absence; 0= no, 1= yes. **10**= stem length; 0= 14cm, 1= 14 < M < 24cm, 2= 24cm. **11**= bract length; 0= 7mm, 1= 7mm < G < 13mm, 2= 13mm. **12**= corolla length; 0= 0 14mm, 1= 13 < 0 < 22, 2= 22mm. **13**= stamen insertion; 0= 3mm, 1= > 3mm. **14**= lobes of the lower corolla; 0= rounded-ovate, 1= ovate, 2= acute-acuminate. **15**= corolla divergence; 0= straight, 1= strongly divergence. **16**= flower regularity; 0= 5 lobes, 1= 2 lipped. **17**= filament villosity; 0= filament glabrous below, 1= filament hairy below. **18**= stem hairy; 0= glabrous, 1= hairy. **19**= Seed coat ornamentation; 0= fibrillar, 1= smooth, 2= pitted, 3= papillate. **20**= Pollen sculpture; 0= microreticulate, 1= verrucate, 2= scabrate-perforate, 3= interweaving-rugulate, 4= rugulate, 5= scabrate.

II.9

A NEW APPROACH TO *Orobanche* SPECIES IDENTIFICATION

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ABSTRACT

As a result of the lack of photosynthetic activity, the Orobanchaceae plastid genome has evolved under conditions of reduced selective pressure. This has led to a large variability between species whereas all the individuals within a definite species exhibit an identical plastid DNA. A large number of individuals belonging to several *Orobanche* species* or supposed species were collected for DNA analysis and particularly for a comparison of their *rbcL* gene. Based upon RFLP analysis of this gene we can easily distinguish species that belong to different subsection (*O. caryophyllacea, O. rapum-genistae, O. gracilis*). This is not the case of the subsection *Minores*, since among four supposed species (*O. minor, O. amethystea, O. hederae* and O. *loricata*) only two groups can be evidenced with molecular characters. One morphological character, the stamen filament colour, also separates the four so-called species in the two same groups. However, *O. hederae* exhibits another morphological difference in comparison with the other specimens of the subsection *Minores* : the yellow colour of the stigma.

* taxonomic names according to *Flora Europaea*

Additional key words : plastid DNA, rbcL, taxonomy

Introduction

According to Musselman (1994), taxonomy is the most neglected aspect of the biology of *Orobanche* in spite of its value for scientists concerned with their control. Taxonomists are faced with considerable variations in morphological characters whose number is largely reduced by holoparasitism. Modern methods of taxonomy have been used during the last few years, including pollen morphology (Abu Sbaih *et al.*, 1994), seed micromorphology (Abu Sbaih and Jury, 1994) and chemotaxonomy (Andary, 1994 ; Georguieva and Edreva, 1994). Unfortunately, these attempts have not been entirely successful and with some of the criteria distinction between some *Orobanche* subsections is not yet possible. This is particularly the case of the subsection *Minores* to which *O. amethystea, O. minor and O. hederae* belong. In recent years, molecular criteria, such as the nucleotide sequence of genes encoding ribosomal RNAs, have been widely used in phylogenetic studies. Such criteria have also been used in some taxonomic studies, particularly in the case of restriction endonuclease analysis of chloroplast DNAs. Such analyses have revealed that related taxa, even within the same genus, exhibit variations (Palmer *et al.*, 1983). The main advantage of this method lies in the fact that individual variations among members of one species very seldom happen, in contrast with molecular analysis of the nuclear genome. Moreover, the plastid chromosome is particularly convenient for such a study since its length is approximately 10^{-5} to 10^{-7} that of the nuclear DNA. The plastid DNA of holoparasitic plants has recently been shown to be both reduced in size and largely divergent in its gene content from those of autotrophic plants. This is due to the evolution of such a plastid genome under the conditions of reduced selective pressure. Consequently restriction endonuclease analysis of plastid DNAs in holoparasitic plant could be an interesting tool to investigate the taxonomy of *Orobanche* whose identification on morphological characters is known to be somewhat uncertain. In the present work our goal was to seek easily applicable plastid DNA related molecular criteria that would allow us to identify *Orobanche* species. The ultimate aim is to marry morphological characters with those obtained from molecular analysis.

Material and Methods

Collecting the Orobanche and selecting the morphological characters. *Orobanche* specimens were collected in the West of France during spring of years 1994 and 1995. Before DNA extraction and molecular analysis, morphological characters were carefully studied. During the 1994 harvesting period, all samples were checked for numerous morphological characters (49) used to establish the determination keys in several flora. A first comparison of variability of the specimens of 1994 according to molecular analysis on the one hand and to morphological variations on the other was established. This allowed us to select a lesser number of morphological characters for the second year of collection although some other were checked only in 1995. These characters are listed in table 1.

DNA analysis. Total DNA from 2 to 3 g of inflorescence of each specimen collected was extracted in liquid nitrogen following the method of Doyle and Doyle (1990). Genes to be sought were amplified by polymerase chain reaction (PCR) as previously described (Thalouarn *et al.*, 1994). Either total DNA or PCR products were digested with restriction enzymes purchased from Boehringer Mannheim under conditions recommended by the manufacturer. Total DNA restricted with endonuclease BamHI was hybridized with a tobacco plastid DNA fragment (pTB18 probe) produced by partial digestion with BamHI, cloned in pBR322 (kindly provided by Dr. Sugiura). Restriction patterns obtained from *rbcL* gene digestion or from Southern blot using pTB18 probe were shown to differ between specimens. In each case (several restriction enzymes were used) two or more restriction patterns were observed and numbered from 1 to n. The *rbcL* gene of each specimen could therefore be described by a six-figure number (e.g., 1 1 2 1 2 4 with 1 for rbcL obtained by PCR, 1 for RFLP type obtained with EcoR I, 2 for RFLP with Pst I, 1 for RFLP with

Alu I, 2 for RFLP with Hinf I and 4 for RFLP with Nde II).

The random amplified polymorphism DNA polymerase chain reactions (RAPD-PCR) were carried out in a 25l volume of 1x Taq Buffer (Eurogentec) containing 15 ng of primer, 25 ng of genomic DNA template, 200 M each of dATP, dCTP, dGTP and dTTP, 4 mM $MgCl_2$ and 0.5 units of Taq polymerase. A single 10-mer oligonucleotide primer (A4 of Operon Technologies Inc., Alameda, CA) was used in each PCR amplification. The PCR was performed in a thermal cycler (Perkin Elmer Cetus). The standard reaction consisted of 45 cycles each of 1 min at 94º C, 1 min at 36º C and 2 min at 72º C.

Results

The morphological analysis conducted on specimens collected in 1994 led us to conclude that *Orobanche* identification is difficult, particularly in the subsection *Minores* to which *O. hederae, O. minor, O. loricata* and *O. amethystea* belong. The species belonging to other subsections are more easily identified. Molecular analysis of the *rbcL* gene showed that the species can be characterized by a specific three-figure identification number (Table 2). However *O. caryophyllacea* and *O. rapum-genistae* which are morphologically different exhibit the same number ; 101. On the other hand, *O. arenaria* and *O. gracilis* whose *rbcL* gene is not amplified have obviously the number 000, although they differ by another plastid DNA characteristic : the *atpB* gene is amplified from *O. gracilis* and not from *O. arenaria* (data not shown). In the *Minores* subsection the specimens pre-identified as *O. hederae* and *O. minor* exhibit a different number, 112 and 113 respectively, whereas among the supposed *O. amethystea* which differ morphologically from one another we found specimens with either 112 or 113.

In view of the 1994 results, in 1995 we focused our objectives on the species belonging to the *Minores* subsection. Restriction sites in the *rbcL* gene were sought with three other endonucleases : Alu I, Hinf I and Nde II. Specimens studied were therefore described by a six-figure number (Table 2). RFLPs of the *rbcL* gene of *O. caryophyllacea* and *rapum-genistae* obtained with Alu I, Hinf I or Nde II were seen different in each case. *O. hederae* samples are all characterized by 112144, whereas *O. minor* are described by 113233. The specimens roughly identified as *O. amethystea* are characterized by 112144 or by 113233. Moreover a specimen identified as *O. loricata* according to morphological characters also belongs to the 113233 group. Consequently the subsection to which *O. hederae, O. amethystea, O. minor* and *O. loricata* (=*O. picridis*) belong seem to consist of two groups 112144 and 113233. In spite of several other attempts (*rbcL* EcoR I/Hinf I double digestion, RFLP of the pTB18 fragment of the plastid DNA) it was not possible to find another repartition of the specimens studied (Table 3). Although there are many morphological differences among them, they obviously belong to two species.

These findings led us to study carefully the morphological characters. Only one of them, the colour of the filament of stamen, is of a peculiar interest since it fits perfectly with the observed differences in the molecular characteristics. All the specimens numbered 113233 (supposed *O. minor, O. loricata* and a part of the *O. amethystea*) exhibit a white filament whereas in the 112144 (*O. hederae* and the other *O. amethystea*) the filament is purple, at least at the top. However the case of the *O. hederae* specimens is somewhat different since on the one hand RAPD patterns are variable in contrast with the other species (data not shown) and on the other the colour of the stigma (yellow) differs from the others (dark purple).

Discussion and Conclusion

The difficulties to identify closely related *Orobanche* species led us to apply plastid DNA related molecular criteria. Using morphological characters, species belonging to different sections (*Trionychon* or *Orobanche)* or subsections such as *O. ramosa, O. caryophyllacea, O. rapum-genistae,*

O. gracilis can be identified or at least regarded as different. This is confirmed by the molecular approach, thus proving its validity. However the main interest of this approach would be as to provide a powerful tool for identification among the species belonging to the same subsection. For this reason we have focussed our studies on the subsection *Minores* which is known to be the most controversial subsection of the genera. In a previous attempt, Chater and Webb (1972) broke *O. minor* into a ten taxa group devoid of formal taxonomic standing. The extreme individual variability in morphological characters among the individuals belonging to the species of that subsection make the identification very difficult , particularly for *O. loricata*, *O. amethystea*, *O. minor* and even *O. hederae*. According to Musselman *et al.* (1982), this may be due to their strongly autogamous character, a process which is suggested as a possible explanation for the numerous races that have arisen in these species. It is therefore not surprising that only two groups can be distinguished among these species after molecular analysis. The most surprising finding is that one morphological character, the stamen filament colour separates the four so-called species in the two same groups. In conclusion, we do not claim that the four species do not exist, but we do believe that those divisions in the subsection *Minores* remain to be demonstrated whereas the division in two groups that we have evidenced, is found on objective criteria since two plants exhibiting different plastid genomes cannot belong to the same species. Moreover the evolution of parasitic plastid genomes under a reduced selective pressure has increased their variations. This is another reason that led us to believe that there are more likely two species than four among the specimens of the subsection *Minores* we have studied. The new approach of *Orobanche* species identification above described is probably suitable for the other species of this genus and particurlarly for the taxonomic tangles like *O. ramosa* / *O. aegyptiaca* or *O. cernua* / *O. cumana*. Moreover other taxonomic problems related to holoparasitic weeds might be attempted and perhaps solved using this molecular approach.

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Introduction

Orobanche species identification is a major problem in agriculture because of the differences in host preference. However, *Orobanche* taxonomy is a problem for several reasons (Musselman, 1994): First, there is an inherent morphological variability within the plant populations. Second, they are non-photosynthetic, they have no leaves, and they produce only short abnormal roots. In addition, the host may influence the morphology of the plant.

A controversy exists as to the validity of some species. *O. mutelii* F.W. Schultz and *O. ramosa* L. (Chater and Webb, 1972; Feinbrun-Dothan, 1978) are regarded a single taxon by Musselman (1986). Further, the distinction between *O. ramosa* L. and *O. aegyptiaca* Pers. in the field is sometimes difficult due to the inconsistent characters used in keys: plants of *O. aegyptiaca* of the same maternal origin differ in size and morphology when parasitized by different hosts, some of which carrying small flowers that very much resemble *O. ramosa* (Musselman, 1986; Joel, unpublished results).

O. cernua Loefl. is known in two different forms. One attacks sunflower, the other attacks vegetables (Kleifeld and Herzlinger, 1984, Parker and Riches, 1993). Whereas these forms are regarded by some researchers as variants of *O. cernua* (e.g. Chater and Webb, 1972) others separate them into two species: *O. cumana* Wallr. and *O. cernua* Loefl., respectively (Joel, 1988), based on both morphological and behavioral characters.

Orobanche population genetics was discussed earlier (Castejón-Muñoz *et al., 1991;* Moreno *et al.,* 1979; Musselman, 1986, 1994; Verkleij *et al.,* 1986, 1991a,b; Verkleij and Pieterse, 1994). Some information on species polymorphism was gained from isozyme analysis of *O. crenata* and *O. cumana* (Castejón-Muñoz et al., 1991; Verkleij *et al.,* 1991a,b; Verkleij and Pieterse, 1994). However, isozyme markers may be affected by environmental conditions and are expressed deferentially at different stages of development.

DNA-based markers are not dependent on environmental and developmental factors and have been applied successfully to discriminate between individual genotypes. The random amplified polymorphic DNA (RAPD) technique (Williams *et al.,* 1990), based on the use of short primers of arbitrary nucleotide sequence in the polymerase chain reaction (PCR), has been useful for DNA fingerprinting (Keil and Griffin, 1994) and can be used for the estimation of genetic relationships within and between species.

In this paper we present the results of a study demonstrating the potential of RAPD markers as a reliable tool for *Orobanche* species identification.

Material and Methods

Orobanche plants used in this study were collected in the field, or raised in the greenhouse from seeds collected in the field, or grown *in-vitro*.

Freshly harvested flower buds were stored at -80°C until used. *Orobanche* genomic DNA was prepared after Fulton *et al.,* (1995). RAPD analysis was performed according to Williams *et al.,* (1990) using ten-mer arbitrary primers. The primers were obtained from (a) the University of British Columbia (designated UBC followed by serial number) and (b) Operon Technologies Inc. (designated OP followed by number). Taq DNA polymerase was used at 0.5 unit in each sample. Amplification products designation included both the primer name and estimated size of the product in bp. 31 ten-mer arbitrary primers were used to amplify DNA of samples from five agricultural important *Orobanche* species.

Southern hybridization of RAPD gels was performed as described by Sambrook *et al.,* (1989). The probes were prepared by excising the amplification products from the relevant lane in an 1.4% agarose gel, and purifying them using the Genclean II kit. ^{32}P Radiolabeling was performed with the *redi*prime™ random primer labeling kit.

Using the PAUP 3.1.1 program (Swofford, 1993) we analyzed the RAPD data accumulated for the different species of *Orobanche*, based on 86 clear polymorphic bands obtained with ten of the 31 examined primers. Polymorphic RAPD bands were treated as a binary (present / absent) character.

Results

The diagnostic information obtained from these seven products is summarized in Table 1. Seven of the 86 polymorphic amplification products were found useful for *Orobanche* taxonomy. UBC 212-300 and UBC300-600 can be used to distinguish between sect. Osproleon and sect. Trionychon of the genus *Orobanche*. Five other bands are useful for the identification of the different species. PAUP analysis of the polymorphic characters produced a single most parsimonious tree (Fig. 1) that separates the species belonging to sec. Trionychon from those belonging to sec. Osproleon, discerns *O. aegyptiaca* and *O. ramosa*, distinguishes between *O. crenata* and *O. cernua*, and shows up *O. cumana* as an autonomous entity near *O. cernua*.

An example of a RAPD pattern obtained with one primer (UBC215) used to amplify three individual plants (grown on different hosts or in different places) of each of the five species is presented in Fig. 2. This primer provided one band (UBC215-470) unique to *O. aegyptiaca* and another band (UBC215-1400) unique to *O. crenata*. In order to improve the visualization and to avoid ambiguity, RAPD patterns obtained with each of the informative primers were blotted and hybridized to the relevant probe (only UBC215-1400 is presented). The hybridization patterns were clearer and much easier to interpret than the RAPD patterns (Fig. 2). Fig. 3 shows the RAPD pattern obtained by the primer OPG6 in a similar sample of plants. OPG6 provided one band (OPG6-400) unique to *O. aegyptiaca*, and another band (OPG6-660) unique to *O. cumana* ,which was confirmed by hybridization.

Two unique bands were found to distinguish *O. aegyptiaca* from *O. ramosa*. The informative primers were tested on 24 individuals representing eight different populations of *O. aegyptiaca*, compared with 24 individuals from four different populations of *O. ramosa*, some of which were parasitizing various host plants. The unique bands OPG6-400 and UBC215-470 were amplified by all 24 tested *O. aegyptiaca* individuals and not by any of the tested *O. ramosa* individuals. An example of the latter is shown in Fig.4, where individuals of three different populations of *O. aegyptiaca* (Fig. 4, A,B,C) and of three different populations of *O. ramosa* (Fig 4, a,b,c), each grown on three different crops as host plants, were examined using UBC215. The marker UBC215-4700 was amplified by all *O. aegyptiaca* individual, not by any of the *O. ramosa* individuals. One plant grown from a stock of *O. ramosa* seeds (lane 10 ,Fig 4) was identified as *O. aegyptiaca* according to the hybridization pattern of UBC215-4700, and indeed all its morphological features were characteristic of *O. aegyptiaca*. This particular DNA sample was also hybridized using OPG6-400, again confirming its identification as *O. aegyptiaca*.

RAPD patterns were similar in *O. ramosa* collected in agricultural fields closely resembles the populations of this species in various native habitats (Figs. 2,3). These populations could easily be distinguished from *O. aegyptiaca* by using the DNA probes that respectively hybridize to each of these species. In a similar manner RAPD patterns of *O. cernua* collected in agricultural fields closely resembled the RAPD patterns of samples of this species collected in a native habitat (Fig. 2).

The informative primers were also used to amplify DNA of samples obtained from other countries. Three samples of *O. crenata* from Egypt are presented in Fig. 2, and three samples of *O. cumana* from Bulgaria are presented in Fig. 3. Interestingly, The unique RAPD and hybridization bands that were found to identify the different species in Israel were also produced by the DNA of the Bulgarian and the Egyptian plants of the same species.

Discussion

The potential of the use of DNA markers of the genus *Orobanche* is clearly demonstrated in our study. The difference between the agriculturally important taxa can be shown using RAPD products, and more so with specific DNA probes for Southern hybridization to RAPD products. Hybridization is very important especially in species diagnostics, because parallel RAPD bands of a specific primer are only similar in molecular size, whereas parallel hybridization bands are similar also in molecular sequence. The diagnostic information obtained from RAPD products, which is summarized in Table 1, is valuable for the study of *Orobanche* in agricultural areas. Probes are now available for most relevant species. Using RAPD primers we were able to confirm the separation of species belonging to section Trionychon from species belonging to section Osproleon and to characterize problematic species.

O. ramosa and *O. aegyptiaca* are often difficult to separate in the field due to inconsistent characters used in keys (Musselman, 1986). *O. aegyptiaca*, when grown on some host plants, can develop into stunted plants resembling *O. ramosa* in their morphological characters (unpublished results). RAPD analysis easily overcomes this difficulty, clearly distinguishing between these two taxa. The difference between *O. aegyptiaca* and *O. ramosa* was shown using a variety of primers, and in a more accurate manner using specific DNA probes of each species for Southern hybridization of RAPD products.

In a similar manner *O. cumana*, that occurs only in agricultural fields, could easily be distinguished from the various populations of *O. cernua*, occurring both in agricultural fields and in native habitats in different parts of Israel. The distinction between these two species is highly relevant because they attack different crops, and should be identified accordingly for the benefit of the farmers. The validity of our RAPD markers obtained for *Orobanche* species in Israel was also tested for *Orobanche* of the same species in other countries. The markers for *O. cumana* and *O. crenata* respectively hybridized with samples of *Orobanche* collected in Bulgaria and Egypt. Samples from other countries are now under examination in our lab.

Acknowledgments

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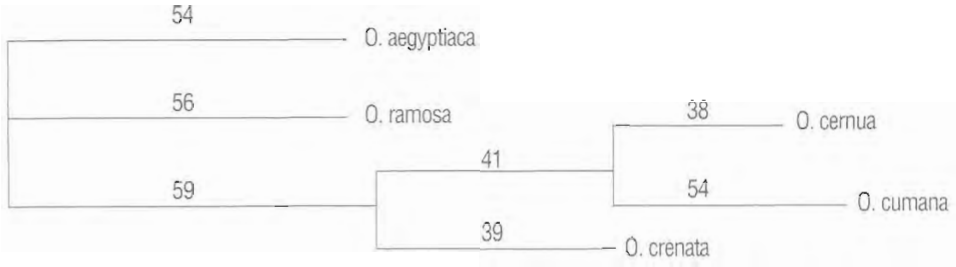

Figure 1

Tree based on parsimony (PAUP) analysis of the 86 RAPD characters of five *Orobanche* species (tree length=341, CI=0.619, RI=0.386).

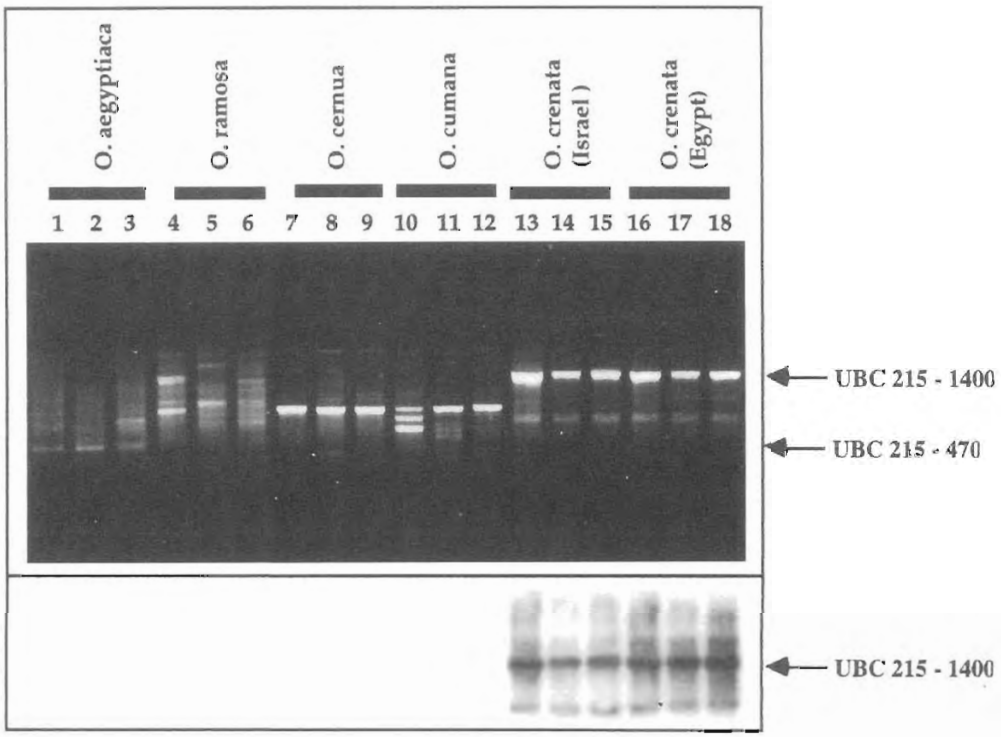

Figure 2

RAPD pattern nobtained with UBC215, of individual *Orobanche*. UBC215-470 is unique to *O. aegyptiaca*, UBC215-1400 unique to *O. crenata*. Patterns obtained with UBC215-1400 were blotted and hybridized to the relevant probe. *O. crenata* from Egypt show the markers typical of the Israeli samples. Lanes 5 and 7: *Orobanche* from natural habitats.

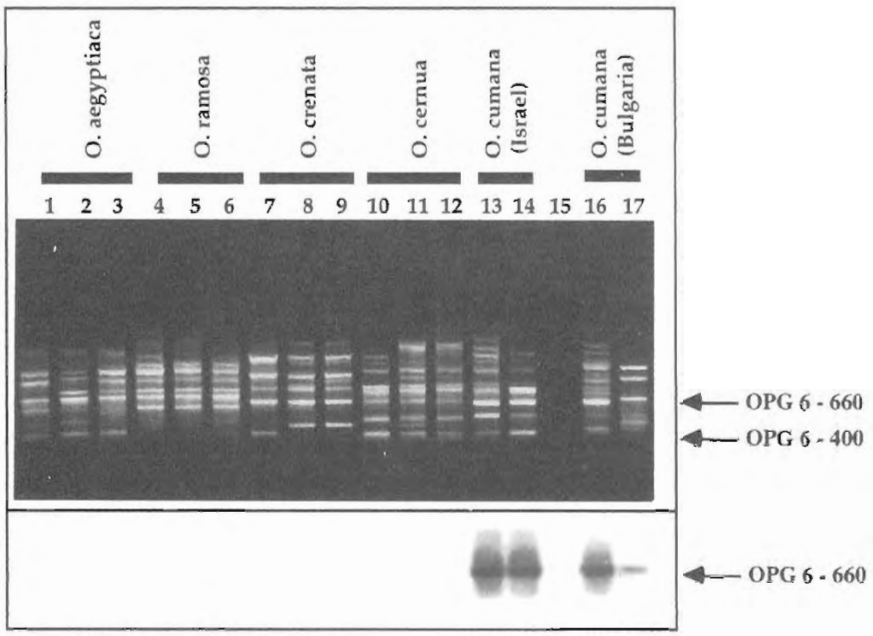

Figure 3

RAPD pattern obtained with OPG6, of individual *Orobanche* plants. OPG6-400 is unique to *O. aegyptiaca* and OPG6-660 unique to *O. cumana* . Patterns obtained with OPG6-660 were blotted and hybridized to the relevant probe. *O. cumana* from Bulgaria show the unique bands characteristic of *O. cumana*. Lanes 5 and 10 - from natural habitats.

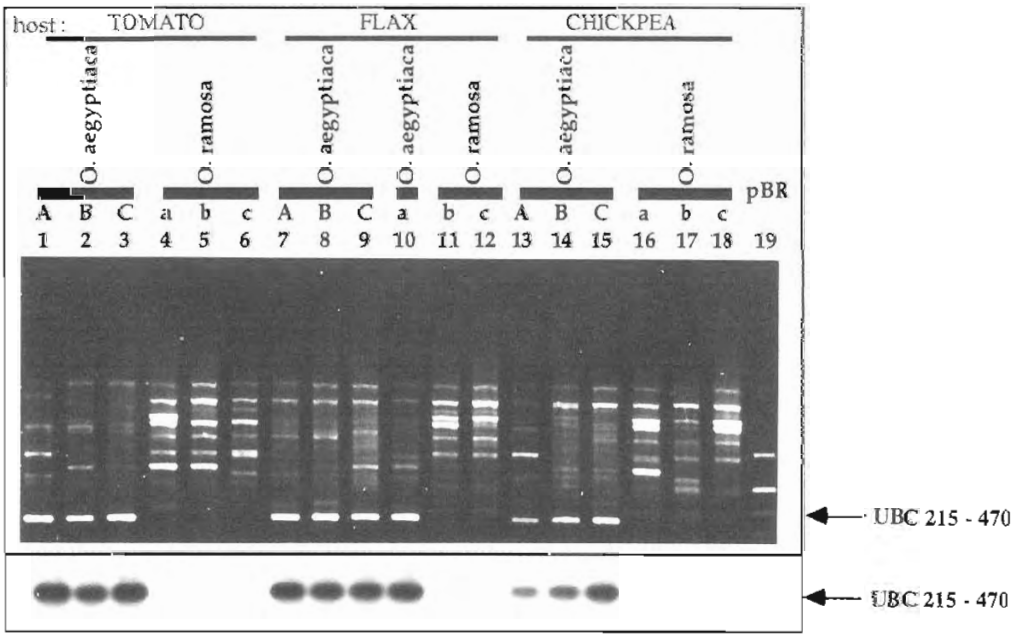

Figure 4

RAPD pattern, with UBC215, of three different populations of *O. aegyptiaca* (A,B,C) and three different populations of *O. ramosa* (a,b,c), each grown on three different crops. UPC215-470 was amplified only by *O. aegyptiaca*.

Table 1

SEQUENCE OF THE 6 OLIGONUCLEOTIDE PRIMERS CHOSEN FOR THE STUDY OF GENETIC VARIATION IN *OROBANCHE CRENATA*

Primer	Sequence (5'-3')	Polymorphic fragments
OPA-02	TGCCGAGCTG	3
OPA-08	GTGACGTAGG	6
OPA-10	GTGATCGCAG	5
OPC-7	GTCCCGACGA	7
OPD-12	CACCGTATCC	8
OPK-17	CCCAGCTGTG	10
TOTAL		**39**

BC215
800

-
-
+

-
-

II.12

Characterization and Differentiation of *Orobanche* Species and Races by Isoenzyme Analysis

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ABSTRACT

The taxonomic identification in certain *Orobanche* species is difficult and race differentiation within a species is frequently impossible just as the distinction of populations which differ in their agressivences or host specificity. Therefore methods for the electrophoretic analysis of isoenzymes were adapted for broomrape species from the section *Trionychon* especially *Orobanche ramosa* L., *O. aegyptiaca* Pers. and *O. mutelii* F. W. Schultz. First results reveal a homogeneity within a population of *O. ramosa* which parasitise tobacco in southern Germany. The other populations under investigation show a polymorphic banding pattern in at least one enzyme staining system. All populations examined can be distinguished from each other.

Key words: *Orobanchaceae*, *Orobanche*, parasitism, host specificity, isoenzyme, electrophoresis, enzyme specific staining.

III.1

Prosopanche (HYDNORACEAE): SOMATIC AND REPRODUCTIVE STRUCTURES, BIOLOGY, SYSTEMATICS, PHYLOGENY AND POTENCIALITIES AS A PARASITIC WEED

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ABSTRACT

An up to date review on the structure and biology of *Prosopanche* is given. The somatic organs are analyzed with regard to morphology and anatomy. The study of reproductive structures covers the sporophytic as well as gametophytic features. New insights on host relationships, pollination and dispersion provide a better picture of its biology. The above data suport a phylogenetic scheme linking *Prosopanche* and *Hydnora* with *Mitrastremon* and Annonaceae.

Key Words: *Prosopanche*, pollination, Hydnoraceae, reproduction.

Introduction

The genus *Prosopanche* has only 2 species and together with *Hydnora* (4 species) it belongs to the family Hydnoraceae. All family members are herbaceous root holoparasites.

Except for the perianth lobes none of the members have leaves or scales of any sort.

The vegetative structures as well as a large part of the flower are completely subterranean; only their perianth lobes and anthers emerge from the soil at anthesis, its inferior ovary remains under the ground until fruit development.

The peculiar nature of the vegetative structure is a matter of conjecture. One idea interprets the somatic body as being formed only by roots, and the other considers it a stem.

Materials are difficult to get due to the furtive nature of these plants and to their seasonal appearance. Up to the present *Prosopanche* appears not to be an agronomic problem; however some indications suggest it as a possible parasite of non conventional crops.

Somatic Structures

The complete absence of leaves, scales and nodes together with the subterranean habit of the somatic structures are suggestive, at first sight, of a root structure. Their anatomical structure is rather complicated and obscured by the presence of large schizogenous mucilage containing ducts (Fig. 2H). So they have been interpreted as roots, and their body is said to be composed of pilot roots (Kuijt, 1969).

However the anatomical structure does not correspond to a root; that is, alternating bundles of phloem and xylem. On the contrary, their bundles are collateral and arranged in a stele with a conspicuous pith. The arrangement of the vascular bundles follows a star like contour rather than a cylindrical one. Large mucilage and tannin containing ducts are located in the pith (Fig. 2H).

The size of the bundles and the mucilage containing ducts is larger in the areas facing the sides of the rhizome cross-sections, but it gradually diminishes as the bundles and ducts approach the angles of the sections, which in fact are the ridges of a three dimensional body. Such ridges are the site of regularly distributed adventitious true primordial roots that may develop intc haustorial structures; they come in contact with a host, or even with another structure of the parasite (i.e. somatic body or a flower pedicel or ovary). The anatomical structure of the adventitious roots are in agreement with what is expected to be a root, that is, a cylinder without pith of alternating phloem and xylem strands.

It is concluded here that the somatic body of *Prosopanche* is an angular rhizome with adventitious roots at its ridges. The material analyzed never showed buds except for flower buds. So apparently there are no secondary branches, the ocasional apearance of dichotomous branches are interpreted as a result of the activity of the meristematic apex.

Interestingly, not a single rhizome has been found with secondary structure in the vascular system. The only secondary structure is a peridermis which develops very early. In fact a primary epidermis is found a few centimeters from the cauline apex. The suberous tannin impregnated peridermis is extensively developed covering the hole rhizome, flower buds, flowers an fruits.

This lack of secondary growth raise a questions because in the field living material is always accompanied by a considerable amount of dried dead material. A fundamental question arises: How long do these plants live?

A perennial condition may be discounted because of the lack of secondary structures in the vascular system, and consequently by the lack of secondary thickening. Then possibilities are that this plants

are either annuals or biennials.Or, are they monocarpic like many bamboos or Agave?

The embryos contained in the seeds persist at the globular stage (Cocucci, 1976; Chodat, 1916), a condition which can be interpreted as thallus-like, since no cotyledons are differentiated nor are root and stem apices. There is no information regarding germination and structure of the seedling. However taking into account the adult structure it seems that the embryonic root apex never differentiates, so that the body starts developing from the stem apex alone, that is, from a meristematic region in which cotyledons have not reached differentiation. This unusual behavior may explain why this bizarre rhizome does not exhibit nodes or leaf or scales.

Reproductive Structures

Due to the fact that *Prosopanche* shares with all Angiosperms the feature of being an haplodiplont with alternating generations, the reproductive structures of the sporophyte and the gametophyte can be considered separately; that is, the flower with its sporangiate organs, as part of the sporophyte, and the sexual organs of androphyte and gynophyte as part of the gametophytes.

3.1. Sprophytic reproductive structures

Andro- and gyno-sporangia are associated in a structure: the flower. Simplification due to fusion and reduction of several flower components, as well as adaptation to pollinators and to the subterranean habit have resulted in a very sophisticated flower of difficult interpretation.

To start with, the flower lacks showy colored appendices, it is covered, externally by a peridermis with suberous tannin impregnated cells guiving it a brown, ferrugineous color. Tannin compounds are profusely distributed all over the flower tissues, and particularly associated with mucilage ducts of lysigenosus nature. Such ducts are longitudinally oriented and are located around the ovary. They play an important role in fruit dehiscence (Fig. 3). Parenchymal tissue is rather abundant in all organs giving them a fleshy consistency. Stone cells form small isodiametrical groups and are lined up 7 to 10 parenchyma strata under the external surface (Fig. 3). Because of this, the flower has a brittle conformation.

The flower pattern is generally trimerous but the tetramerous condition may appear more or less frequently, the dimerous condition being rare. Tepals are fused at the base forming a tube which is also fused with the base of the staminal filaments and the carpels as well. The ovary is completely covered and fused with such a tube, but part of the tube formed by tepals and staminal filaments (the tepalostemon) extends beyond it for few centimeters (Fig.3D).

The androecium is very complicated (Cocucci, 1976). All anthers are fused in a single body comprised of 3, sometimes 4 and rarely 2 groups of 10 extrorse stamens each (Fig. 1B; 2G; 3A,D).This structure, termed here antheral body, almost obliterates the tepalostemon tube. Filaments are fused in 3 bundles that emerge from the tepalostemon rim opposing the tepals, in such a way that the access to the tepalostemon tube is restricted to 3 small holes or windows (Fig. 1B). In an internal whorl, alternating with the filaments 3 bilobed bodies are located,which are interpreted as staminodes (Solms-Laubach, 1894,1901; Cocucci, 1975). Four androsporangia correspond to each anther. Androsporogenesis conforms the successive type of cytokinesis giving rise to generally isobilateral or rarely decussate tetrads (Fig. 4C, F). Androspores are dicolpate the sporoderm exhibiting a non bacculate thick brain-like pattern. Pollen grains, included in an sticky matter are shed by an unusual mechanism at the 2-celled stage. At dehiscence time the anther wall is formed by 2 active layers, the epidermis and the endothecium, plus remnants of mechanically

inactive parietal layers. Both epidermis and endothecium have wall thickenings located in the radial and outer tangential walls (Fig. 4A, B). Although they are of different nature (cutin in the epidermis and lignified fibrous thickening in the endothecium) both behave in the same manner under desiccation. They roll toward the inside, gradually occupying the loculus lumen, and consequently extruding a pasty mass of pollen (Fig. 2C)

Gynoecium organization is no less complicated (Cocucci op. cit.). In fact the carpels, ca. 30 (usually 27) are fused following a parietal pattern in 3 groups. Placentae are laminar, vertically oriented occupying the entire loculus (Fig. 3H). The ovary in sunked into the receptacle, in a way similar to the Cactaceae, but with the striking feature that no foliar remnant of the carpels are left. No leafy carpel is present at the ovary bottom, the sides or the top, nor is a style or a stigma formed. In fact the stigma is formed by the upper part of the placental lamina (Fig. 3D,G).The carpellate whorl may be considered as a nude one reduced only to the placental tissue; the ovary wall would thus be formed solely by the hypanthium.

Ovules are sunk into the laminate placentae to which the integument is fused, only the micropyle being evident (Fig. 5B, E). The nucellus or gynosporangium is completely separated from the integuments and follows a tenuinucellate development pattern (Fig. 5C, D). Gynosporogenesis (megasporogenesis) is of the Allium type.

The gynoecium undergoes great changes as a consequence of the fertilization. Placental tissues grow, their parenchyma cells enlarge and multiply to obliterate the loculus. Placental sheets become fused in a single mass, inside this mass seeds complete their development. The growth of the placental tissue is not accompanied by growth of other floral tissues of the ovary wall, so a more or less irregular transverse dehiscence takes place (Fig. 6)

Gametophytic reproductive structures

The androphyte is a 3 celled individual, as in all Angiosperms. The first division of the androspore occurs inside the androsporangium prior to dehiscence. As is usual the generative cell differentiate parietally and later on it is included within the generative cell cytoplasm (Fig. 4I, J). It has a conspicuous wall of optically active carbohydrates traversed by a large number of plasmodesmata (Cocucci op. cit.). Sperm formation and differentiation is accomplished during pollen tube development.

The gynophyte (embryo sac) (Fig. 5B, F, H) follows the *Allium* type pattern of development resulting in a 7 cell structure with a very well differentiated egg apparatus, 3 very small antipodals and a large highly vacuolate central cell bearing 2 polar nuclei that finally fuse in one while maintaining individual nucleoli (Cocucci op. cit.).

Fertilization and seed formation (Cocucci op. cit.): Fertilization is porogamic and pollen tube entrance takes place through one synergid. The egg cell plus one sperm give rise to the embryo zygote, the other sperm plus the central cell give rise to the endosperm zygote.

The embryo zygote remain undivided for a while, Its resting period ends when the endosperm has a considerable numbers of cells. Embryogenesis follows the solanaceous type but usually it does not prfoceed beyond the globular stage reaching only rarely the quasi trapezoidal (Fig. 5A, I).

The endosperm zygote starts dividing immediately after syngamy. Cytokinesis is accompanied by wall formation following a cellular pattern. First division is by formation of a transverse wall and subsequent divisions are by vertical ones in each of the sister cells. Later, wall orientation becomes irregular. Endosperm cells accumulate proteins, starch and a large amount of other reserve carbohydrate in their cell walls (Fig. 5A). Nucellar cells behave in a similar manner to endosperm cells regarding their

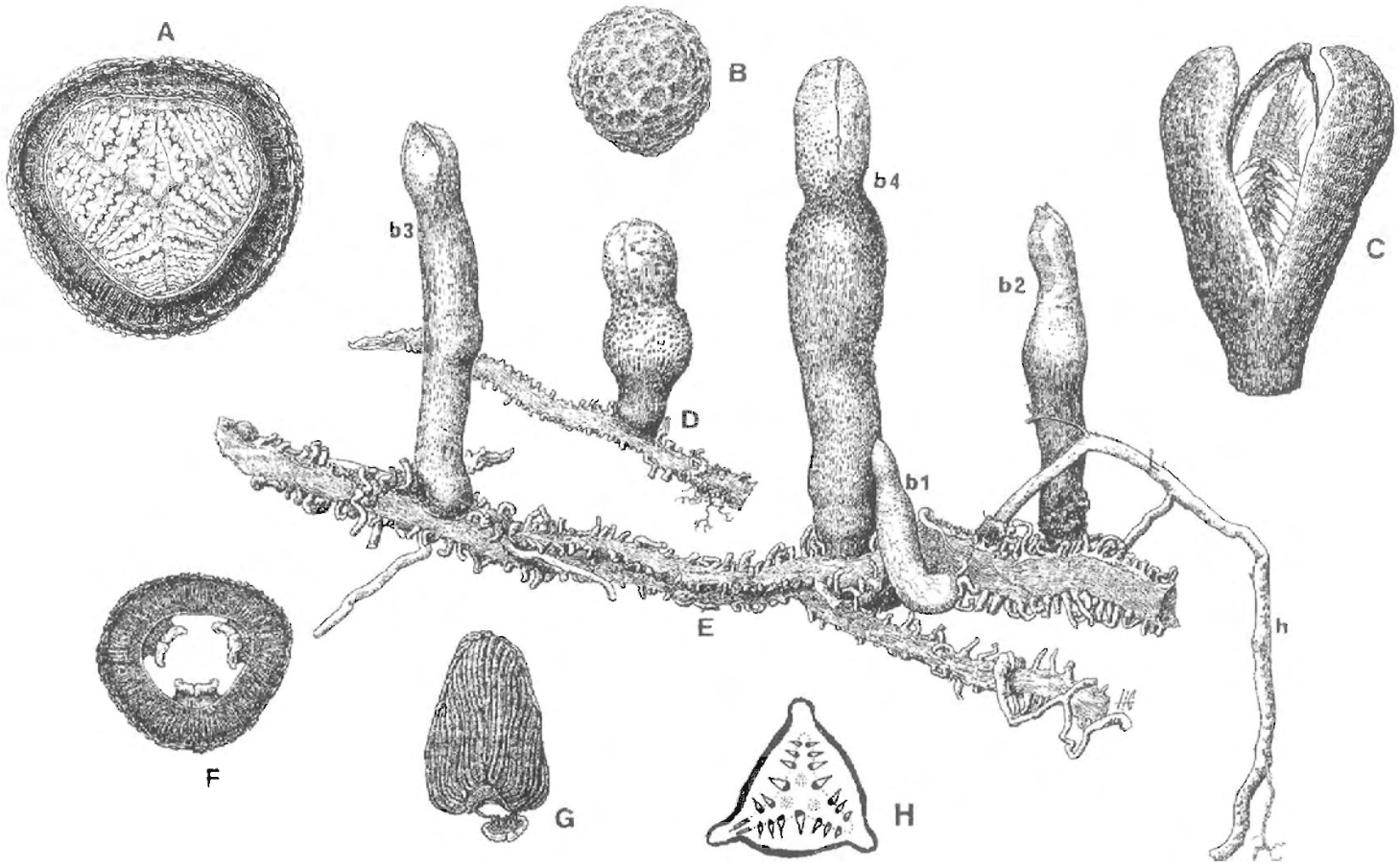

Figure 2

P. bonacinae (From Cocuccu 1965). A, Tepalosemon cross-section showing stigma; B, seed lateral view; C, open perianth showing the antheral body with extruded pollen mass; D rhizome apical end with one flower bud further left; E, 3 angled rhizome with flower buds in progresive stage of development, b1 b2, b3, b4, h root fragment of *Salpichroa origanifolia* connected to *Prosopanche* haustoria; F, tepalostemon cross-section showing staminodes; G, antheral body with one staminodes; G, antheral body with one staminode at the bottom; H, Rhizome crossection, black areas in vascular strands: micilage ducts; A x 5; B x 43; C x 1.4; D, E x 0.72; F-H x 2.

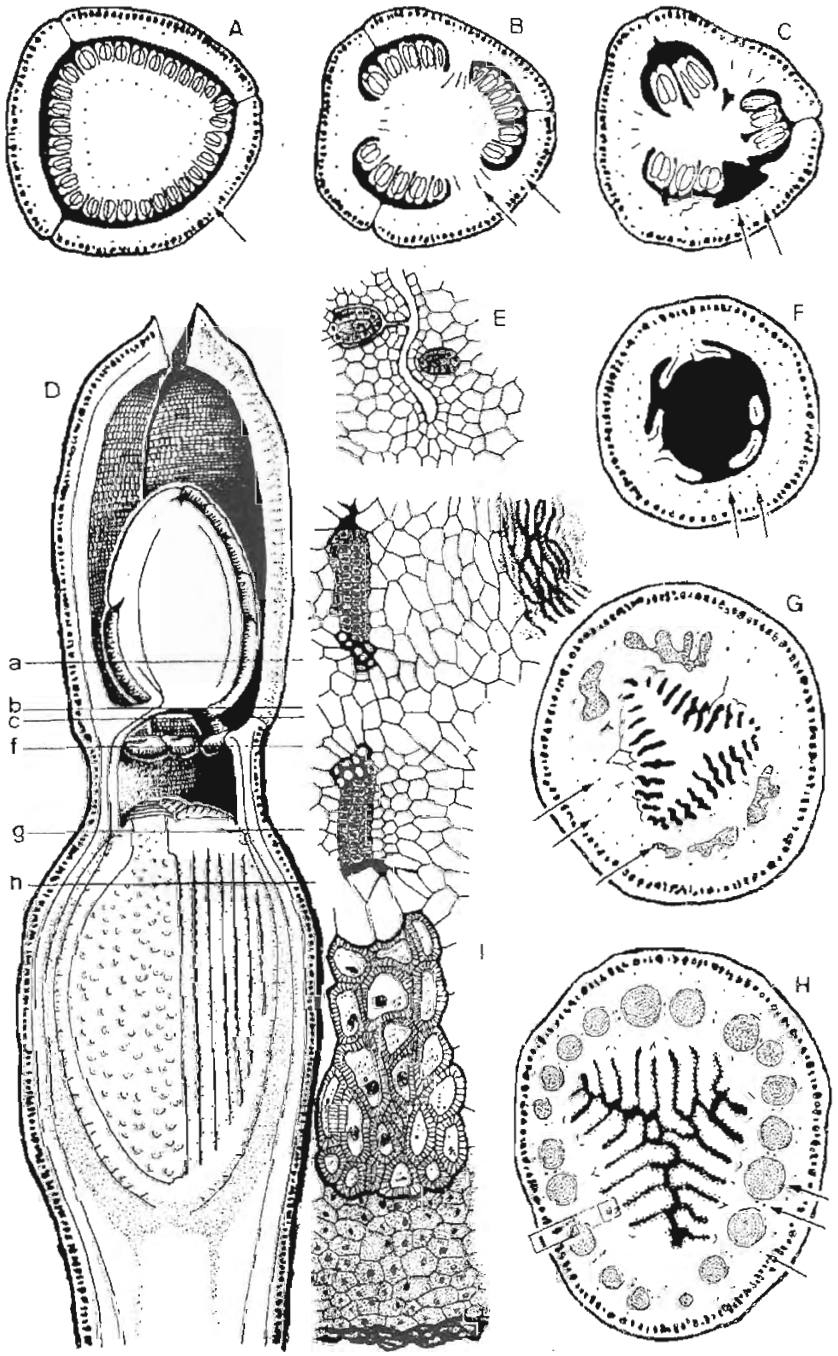

Figure 3

P. bonacinae flower anatomy (From Cocucci, 1975). A, B, C, F, G, H cross-sections at levels indicated a, b, c, f, g, and h in fig D; D, flower longitudinal section prior to anthesis; E, I, details of the areas indicated in H, I, E ovules included into the placental tissue; I from bottom to top, periderm, stone cells group, normal vascular strand, recurrent vascular strand, top rigth part of mucilage duct. Symbology: dotted areas mucilage androecium bundles, in G mucilage duct, perigonial bundle and androecium bundle, in H mucilage duct, normal and recurrent ovary bundles. A-D and F-H x 2.5; E I x 34.

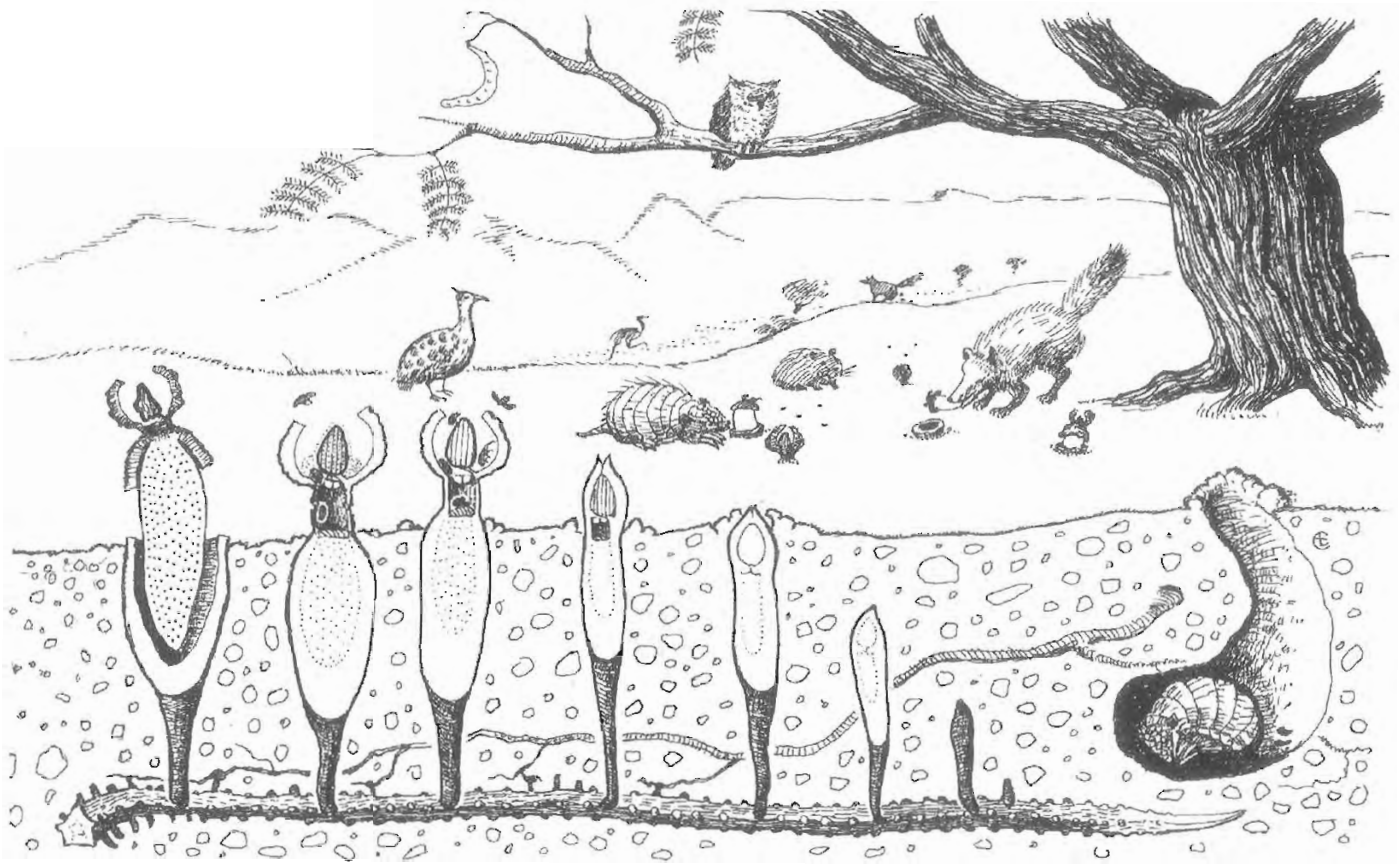

Figure 6

P. americana life history. At the bottom 5-angled rhizome associated to roots of a *Prosopis* tree by means of haustoria. The rhizome exhibits a stem growing point at the wrightend and sequence of progresive devolopment stages from flower bud (wright) to mature fruit (left); stage 6th. a receptive flower attracts to nitidulids and weevils, stage 7th. the same but with antheral body dehiscent and nitidulids leaving the flower, stage 8th. mature fruit. On soil surface different vertebrates probably involved in the zoohcorus disperion; Birds from left to wrigth: "perdiz copetona" (Eudornia formosa elegans), "nandu" (*Rhea americana*) Buho (*Buho virginianus*); Mamals: "quirquincho" (*Chaetophractus vellerosus*), "cuis" (*Microcavia australis*); fox (*Dusicyon gymnocercus*).

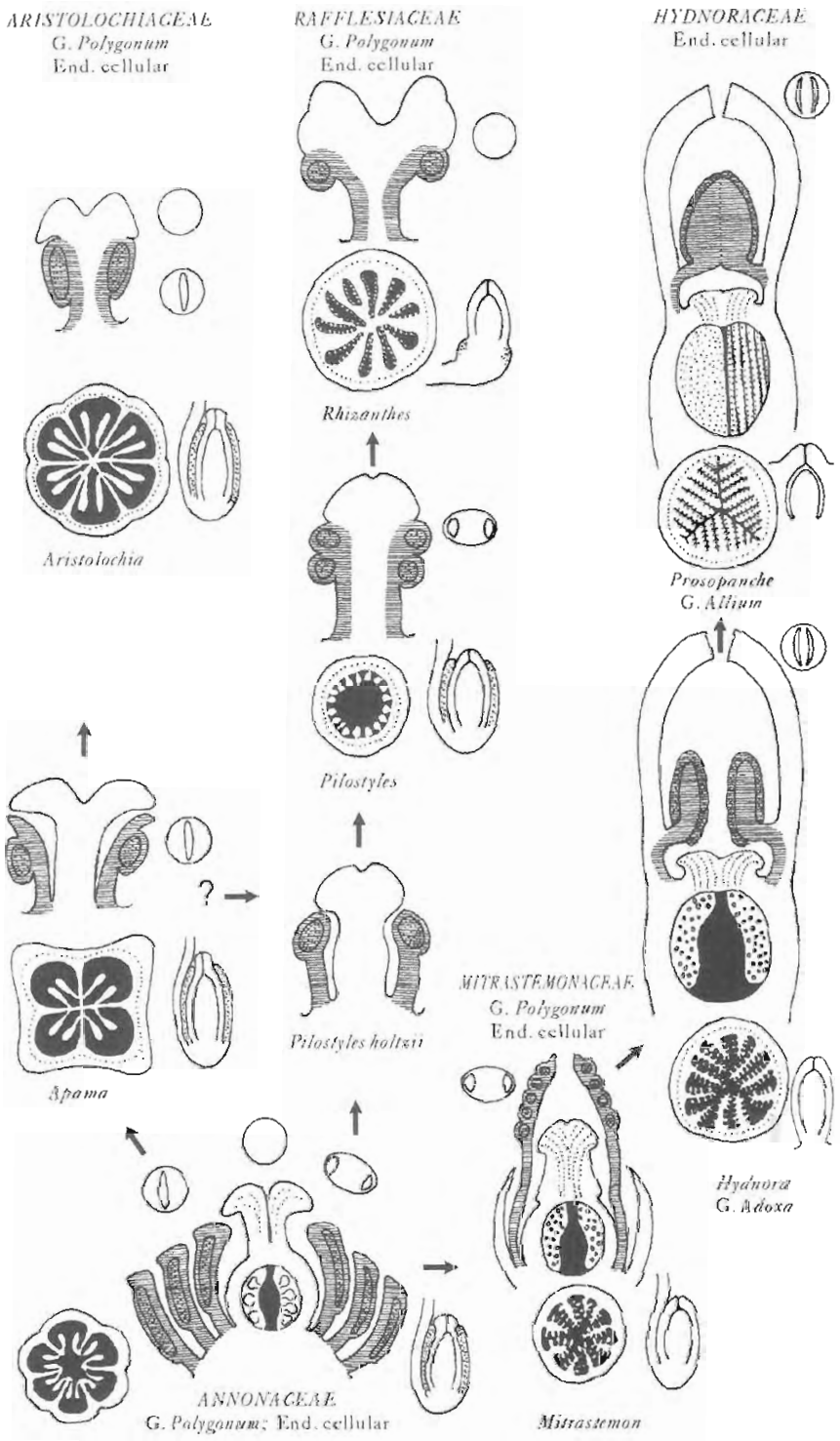

Figure 7

Idealized diagram of possible phylogenetic relationships of *Prosopanche* (From Coucci 1976), explanation in the text. G = gynophyte type; End. = Endosperm.

Introduction

The interface of the haustorium of higher parasitic plants with their hosts is still a gap in our knowledge. But the cellular connection between host and parasite is of central significance as the exchange, presumably especially the withdrawal of substances from the host and consequently the host's main damage, occurs via this pathway. So the occurrence and the fine structure of the interspecific contacts need to be considered when we discuss the phenomena of parasitism (Kuijt 1991). The points of question are interspecific plasmatic connections, the plasmodesmata and sieve pores, as well as the non-plasmatic contacts between the dead xylem elements.

Material and Methods

Results on three host / parasite combinations are reported in this paper:

Striga gesnerioides Willd. (Scrophulariaceae) on *Pisum sativum* L. cv. Kleine Rheinländerin (Fabaceae).

Orobanche crenata Forsk. (Orobanchaceae) on *Vicia narbonensis* L. (Fabaceae).

Striga hermonthica Benth. (Scrophulariaceae) on Zea mays L. cv. Plata.

The plant material was cultivated in culture boxes with a transparent Plexiglas front for better control (Linke and Vogt 1987). Culturing was carried out in controlled environment chambers with 22C for *Orobanche* and 30C for both *Striga* species, relative humidity of 80%, and a permanent irradiance of 300-330 mol m^{-2} s^{-1}. In the appropriate developmental stage, specimens were dissected, fixed and embedded for light- and transmission electron microscopy (Dörr and Kollmann 1995).

The selected embedded haustoria were cut on a Reichert OM U2 ultramicrotome, investigated either in a Zeiss Axiophot light microscope, or a Philips CM 10 electron microscope. For scanning electron microscopy the infected roots were split longitudinally in two parts. Specimens of about 1cm length were left in 70% ethanol for 24h at 6ºC and fixed in FAD (Formaldehyde dimethyl acetate) at 6ºC for 16h. This fixative was evaporated from the specimen by critical point drying in a CPD 030 (Balzers). The roots were affixed to a specimen holder with the split surface upwards and coated with gold and observed with a scanning electron microscope AR 1000 (Leitz) operating at 20kV.

Results

Plasmodesmata

Striga gesnerioides grows on *Pisum sativum* not very vigorously. Nevertheless, both tissues interdigitate strongly. The attacked host root increases its tissue under the influence of the invading parasitic cells with large area of intermingled cells. To identify the exact haustorial interface a discrimination between the cells and their assignment to one or the other partner was necessary. This can be achieved only by cell specific markers. The nuclei and the plastids turned out to be different in the particular *Striga/Vicia* combination. Nuclei of *Striga* were predominantly euchromatic in contrast to those of *Vicia* which appeared heterochromatic (Fig. 1). The plastids of *Striga* (arrows) show dark inclusions and no thylacoids while the host cells have plastids with some internal membrane structures but without electron-dense inclusions (arrowheads). This method allows us to clearly identify the common cell wall. If this wall is investigated closer, numerous plasmodesmata connecting both foreign tissues can be seen (Fig. 2). These plasmodesmata appear predominantly in areas where the common cell wall (of both partners) is especially thin.

Sieve pores

Orobanche crenata grows vigorously on *Vicia narbonensis* and the foreign cells intermingle strongly. Up to this point a complete demarcation line

between both partners cannot be identified as markers for the parenchyma cells so far have not been found. But right at the moment when cells of both tissues differentiate into sieve elements a structural identification is possible. Three different pairs of species-specific markers have been recognized (Dörr and Kollmann 1995). In Fig. 3 the paracristalline body (typical for sieve elements of Fabaceae) marks the *Vicia* sieve element (arrowhead), while the *Orobanche* assimilate conducting cell is recognized by spherical inclusions (arrows) within the plastids. Both specialized cells are connected via sieve pores. The enlargement of the pores of a serial section (Fig. 4) shows two continuous crossings, typically bordered by a callose cylinder, fusing the sieve element protoplasts of *Vicia* and *Orobanche*.

Xylem contact

Xylem contacts of the union between *Striga hermonthica* with *Zea mays* and with *Sorghum bicolor* as well of *Striga asiatica* with *Zea mays* were intensively studied (Dörr in prep.). The scanning electron microscope was especially helpful in revealing the unique structure of this contact. Haustorial cells penetrate mainly the large vessels of the host. They often grow through pits of the xylem element, but can also perforate larger wall parts. The cell protrusions extend brush-like into the lumen of the vessel, the tips open, resulting in cup- and trunk-like structures (Fig. 4). The protoplasts degenerate and the walls become strongly lignified. This open xylem connection leads continuously into the haustorial xylem, consisting mainly of vessel elements. Nearly identical xylem connections have been observed in the two *Striga* species on their monocotyledoneous hosts while those of *Striga* gesnerioides parasitizing dicotyledones differ completely. The same type of open xylem connections have been described by Musselman and Dickison (1975) for *Seymeria* on *Pinus* and for *Aureolaria* on *Ulmus*.

We propose to name the characteristic cup- and trunk-like structures of the parasites exploiting the host xylem "oscula".

Discussion

One of the most important structures of the haustorial interface are certainly all forms of open connections between host and parasite.

A major difficulty is the problem of identification, especially of living cells. In the haustorial area, cells of both partners often are so intensively intermingled that the exact interface can not be easily detected. The disclosure of reliable marker systems for the different cells is a remarkable step forward. For the first time, it was possible to see in *Orobanche* parasitizing the highly compatible *Vicia narbonensis* interspecific sieve pores as well as plasmodesmata from which they derive (Dörr and Kollmann 1995, in contrast to Dörr 1990). In *Cuscuta* the discrimination of interspecific plasmodesmata (Dörr 1968, Dawson et al. 1994) is not a problem as the hyphae are easily to distinguish from the host cells.

In graft connections plasmodesmata and sieve pores between stock and scion have been detected earlier by means of cell markers (Kollmann and Glockmann 1985, 1990). The investigation of other parasitic systems with the aid of cell markers will give more detailed information about open interspecific bridges.

"Open" interspecific connections surely are responsible for a rapid transport of all kinds of substances, either naturally occurring in the plant or for introduced chemicals. With regard to interspecific sieve pores and open xylem perforations, the function in assimilate- and water transport is obvious - especially in the case of holoparasites or parasites lacking their own root system. At present, the significance of interspecific plasmodesmata is still problematic. In addition to function in transport of substances, they are also likely necessary for a synchronous specialization of the foreign tissues and cells.

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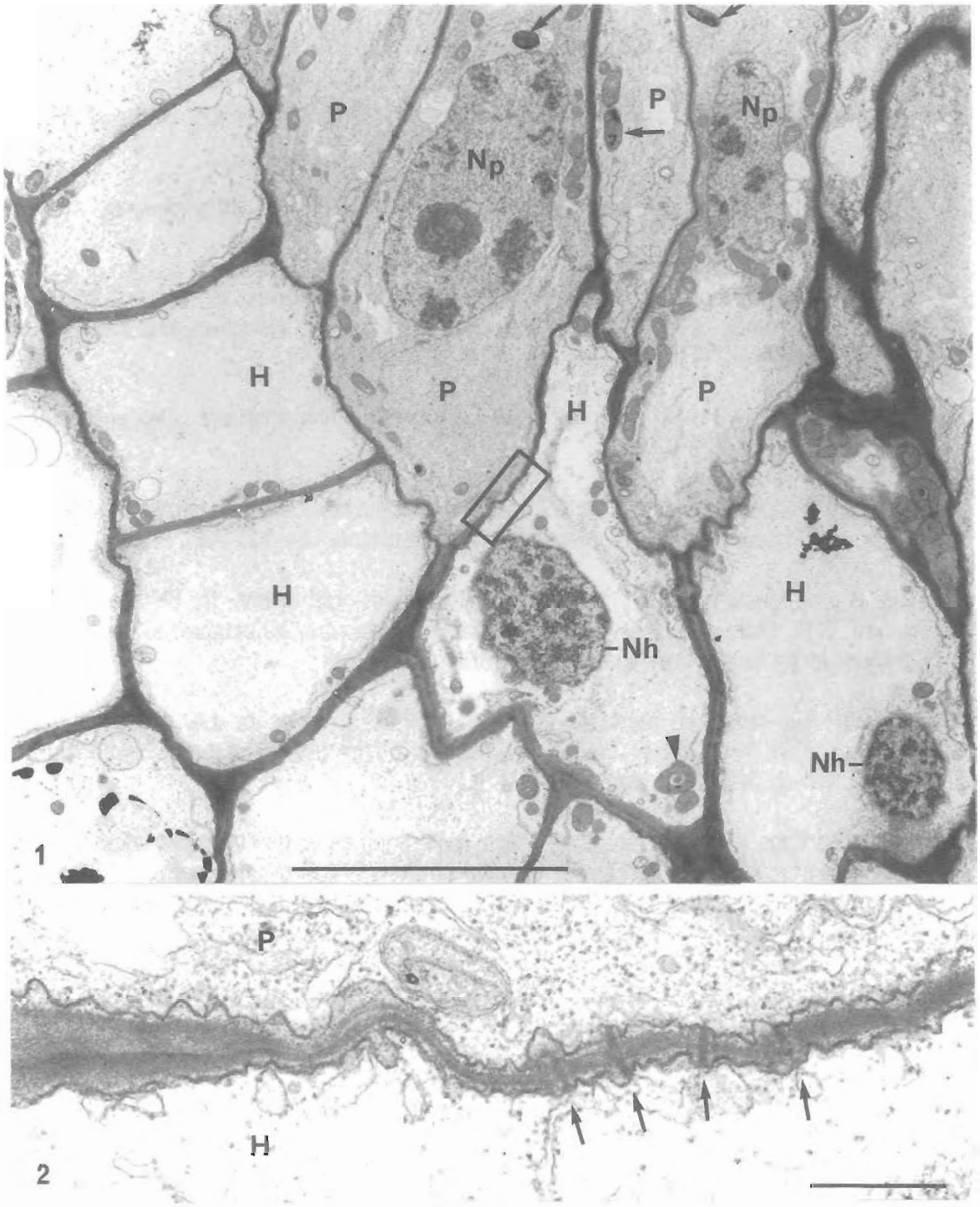
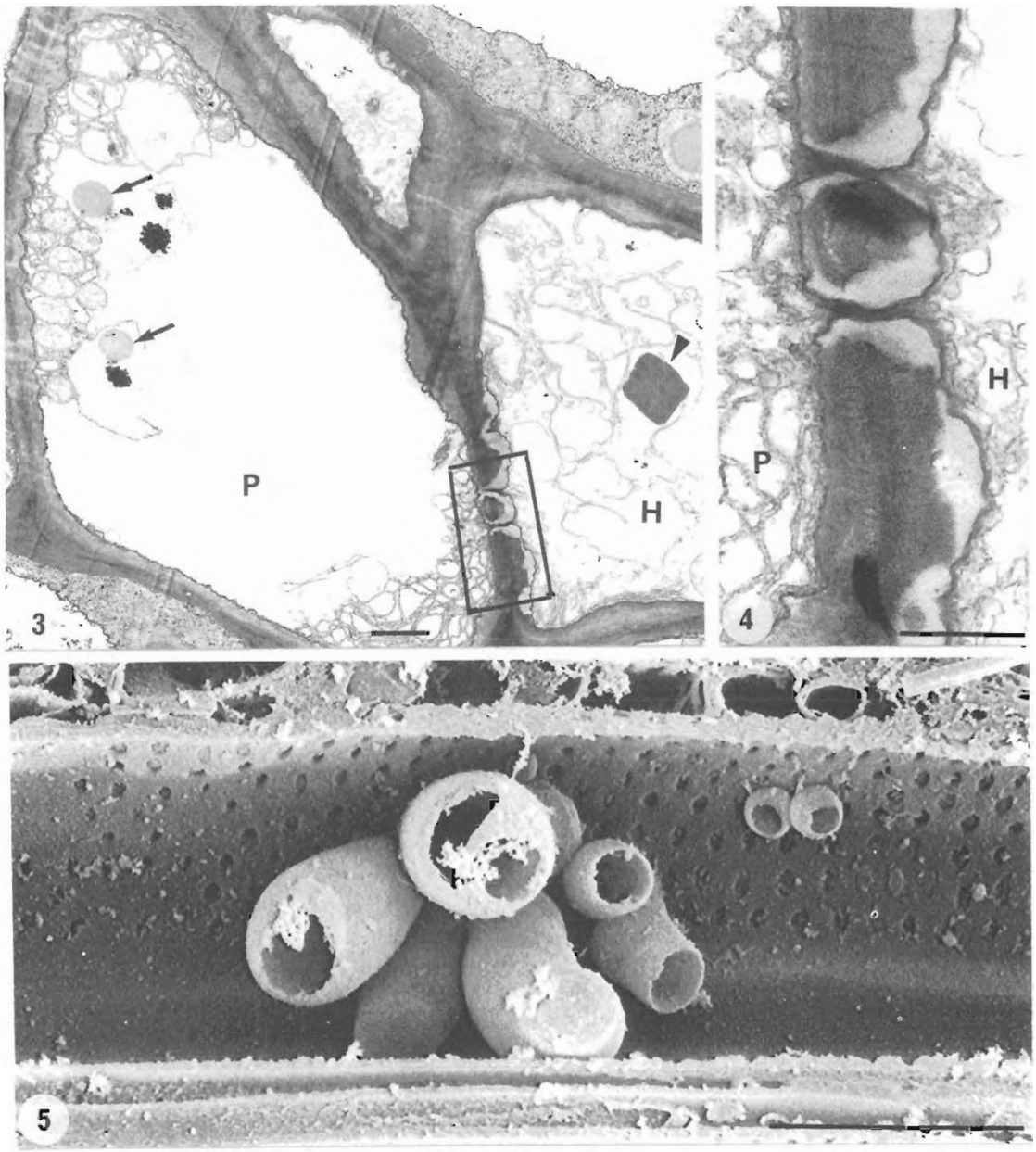


Figures 1-2

Transmission electron micrograph of a part of the haustorial complex (P) of *Striga gesnerioides* parasitizing *Pisum sativum* (H). The interdigitating cells of both partners are discriminated by cell-specific markers. *Striga* shows euchromatic nuclei (Np) while the nuclei of the host (Nh) are heterochromatic. The plastids of *Striga* (arrows) include dark components without thylakoids while those of *Pisum* (arrowhead) show distinct thylakoids without electron dense material. Inset see Fig. 2. X 4,500; bar = 10 m.

Enlargement of the inset of Fig. 1 showing interspecific plasmodesmata (arrows) connecting the protoplasts of the parasite *Striga* (P) and the host Pisum (H). X 44,000; bar = 0,5 m.



Figures 3-5

Transmission electron micrograph of two foreign sieve elements at the haustorial interface of *Orobanche crenata* and *Vicia narbonensis*. The parasitic sieve element (P) is identified by spherical inclusions (arrows) within the plastids while the sieve element of the host (H) shows a typical paracristalline body (arrowhead). Both specialized cells, belonging to different unrelated taxa, are interconnected by continuous sieve pores (inset). X 8,800; bar = 1 m

The area of the inset of Fig. 3 shows in a serial section two continuous sieve pores - here enlarged - interconnecting the both foreign assimilate conducting elements (P = parasite; H = host). X 38,000; bar = 0,5 m.

Scanning electron micrograph of a longitudinally split vessel of the host *Zea mays* with pitted lateral walls. The lumen of the vessel is invaded by cell protrusions of the parasite *Striga hermonthica*. These water-uptaking organs loose their protoplasts during differentiation and show perforated tips. X 3,800; bar = 10 m.

known (Paré and Raynal-Roques, 1992; Paré, 1994; Tykarska and Kuras, 1995).

2. Relation between the rythm of development of the embryo and the growth of the inflorescence

This work was performed on *S. hermonthica* parasitizing sorghum. The zygote is formed 5 to 6 days after anthesis and within 24 h, it is divided by a transverse wall giving rise to a 2-celled proembryo. During the following 24 h, cell divisions lead the T tetrad. Then, 9 days after anthesis, the apical area is a 4-celled globular embryo. During the next 2 days, the proembryo increases in diameter by continued cell divisions and reaches the octant stage of development. These localised divisions within this group of cells lead to the enlargement and the flattening of the globular embryo which reaches the heart-shaped stage 13 to 14 days after anthesis. At this time the hypophysial area is differentiated. Four to 5 days are still necessary for the maturation of the embryo. So, only 16 to 18 days are necessary to form mature embryo (Paré, 1993, 1994). It is noteworthy that the hypophysial initial is differentiated after the division of the apical cell of the suspensor, 10 to 11 days after anthesis.

The rythm of flowering is correlated with the chronological development of fruits, and consequently the development of embryos and seeds, on the same spike.

3. Experiments with 2,4-D

Experiments were performed on *S. hermonthica* in Mali and in Burkina Faso. They were repeated twice. After each experiment, the development thousands of embryos was examined.

Some plants are totally destroyed by the herbicide but, generally it is only the tip of the inflorescence, i. e. the meristematic apical zone, that is the most sensitive to the treatment.

The first formed fruits are not destroyed (Paré, 1993; Ouédraogo, 1995) and give normal embryos (Pl. II, fig. 1) and seeds able to germinate (Ouédraogo, 1995).

The herbicide is the most efficient on the young ovaries and on first stages of development of the embryos, i.e. during a short period occurring just before the fertilization and extending to the formation of the initials of the radicle (Paré, 1994). Similar results were obtained with low and high doses of herbicide.

During the growing period of the crops, new spikes of *Striga* are developing continuously, producing new flowers and new seeds. These seeds can escape the action of a single herbicide treatment, even using a high dose. It appears necessary to carry out several treatments to be sure to destroy all the embryos and thus to avoid any increase in the stock of viable *Striga* seeds in the soil. Moreover, it is important to mention that even if the top of the spikes are destroyed, some lateral buds develop new axes (secondary axes) which can give flowers and fruits (Pl. II, fig. 2).

Conclusion

Our studies show that the precocity of differentiation of the hypophysis and the stability of the embryo structure represent characteristic aspects of *Striga* embryogenesis. This stability reduces the efficiency of chemical treatments.

Moreover, whatever the species, the embryologic pattern of *Striga* follows the *Capsella* type which is one of the most efficient in the Angiosperms.

The correlation established between the development of the spikes and embryogenesis gives a useful field indication to determine the best period of treatment to agronomists and farmers.

To optimize the treatment with 2,4-D, we suggest the schedule reported in figure 1:

- a first treatment carried out when the first flowers of *Striga* appear in the field. This kills the parasite and the seeds at the very young stage of development;
- a second treatment and eventually a third at intervals of 10 to 12 days, to destroy newly emerged *Striga* or regenerated ones developed from axillary buds before fruiting. In this case, lowest doses of 2,4-D are successful.

This protocol implying and at least 2 treatments presents three advantages: (1) it is cheap, accessible to small holders; (2) it is more efficient (3) it is environmentally sound. However, even scientists are confident in this new method of treatment, it remains now to convince the farmers of its efficiency.

ACKNOWLEDGMENTS:

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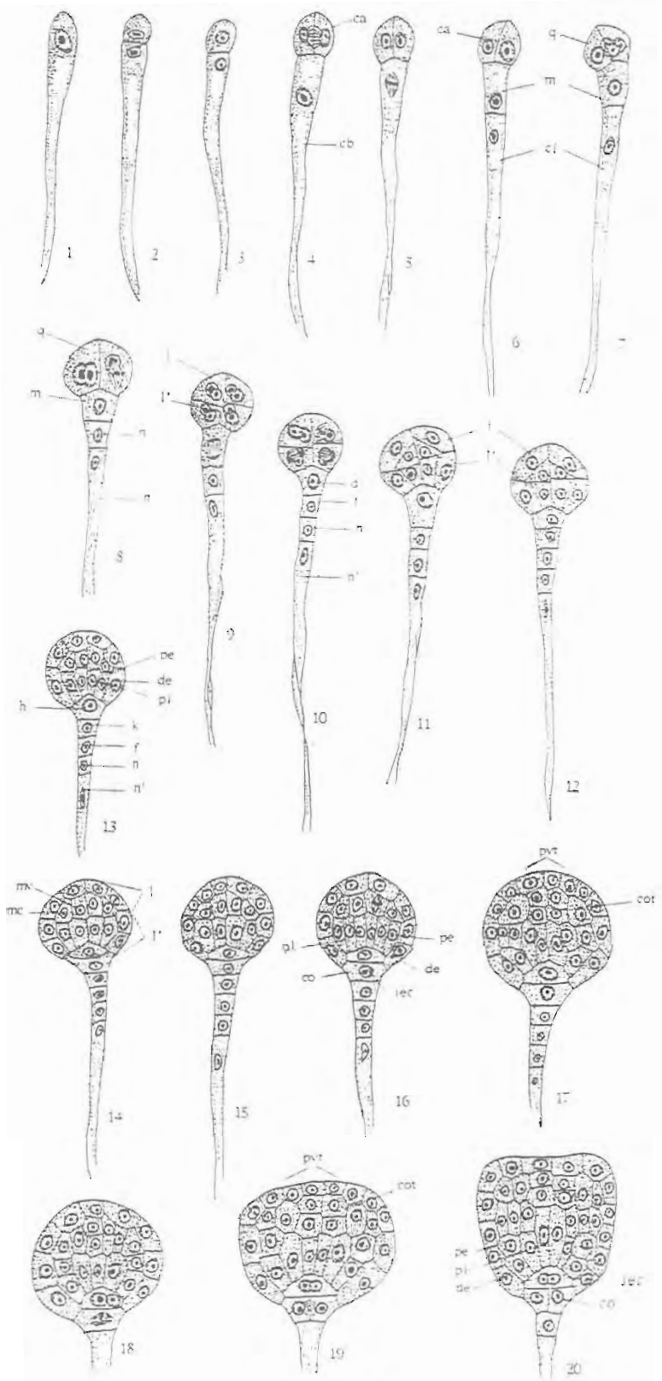

Plate I

Embryogenesis in the genus *Striga*; the most important stages of the development of the embryo: from the zygote to the mature embryo (Gr. X : 360).

ca: terminal cell of bicelled proembryo; cb: basal cell of bicelled proembryo; ci: lower daughter of tetrad; co: cap or central part of the cap; cot: cotyledon initial; d: upper daughter cell of m; de: dermatogen; f: lower daughter cell of m; h: hypophysis; iec: initials of root cortex; m: intermediate cell of tetrad; n: upper daughter cell of ci; n': lower daughter cell of ci; l: upper octants; l': lower octants; pe: periblem ; pl: plerome; pvt: epicotyl or stem tip; q: quadrants.

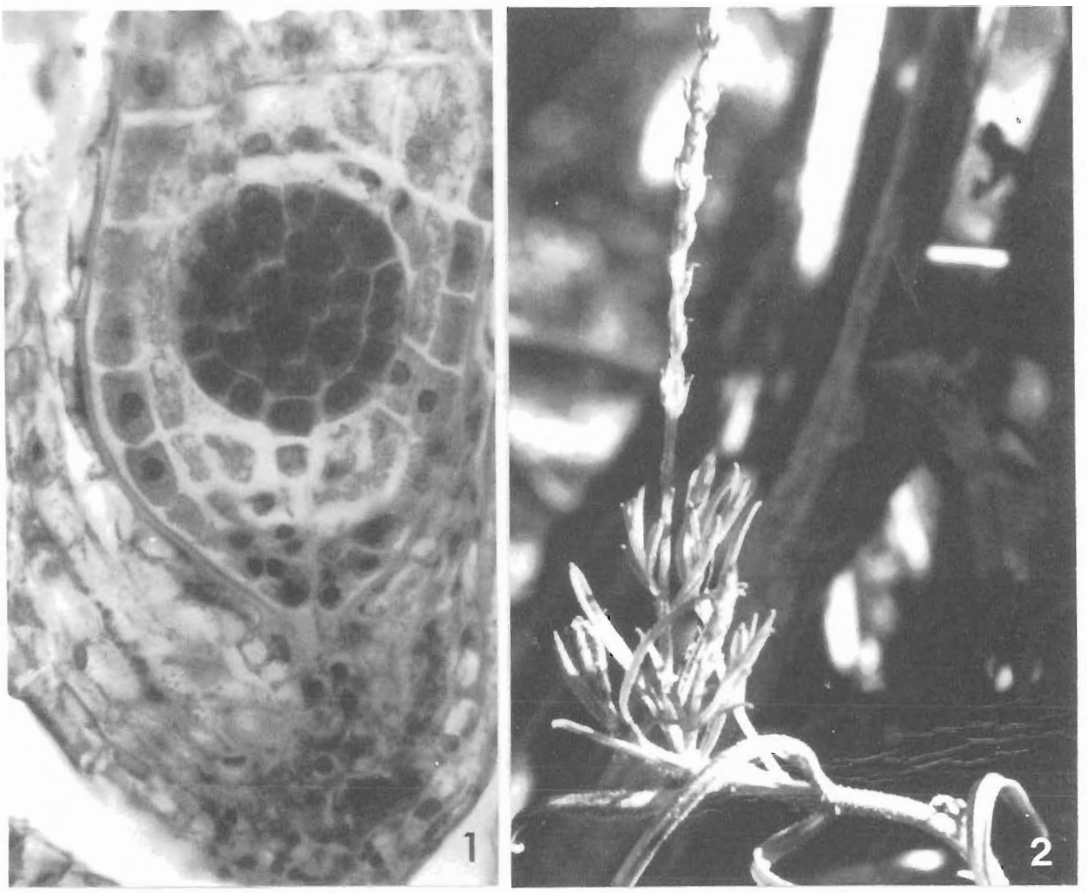

Plate II

Different patterns after action of the herbicide :
1 : Micropylar zone of a seed, with normal globular embryo, in a fruit which has escaped the action of a single herbicide treatment (Gr. X : 430).
2 : New secondary orthotrope axe from a lateral meristematic bud of an inflorescence partly destroyed after treatment.

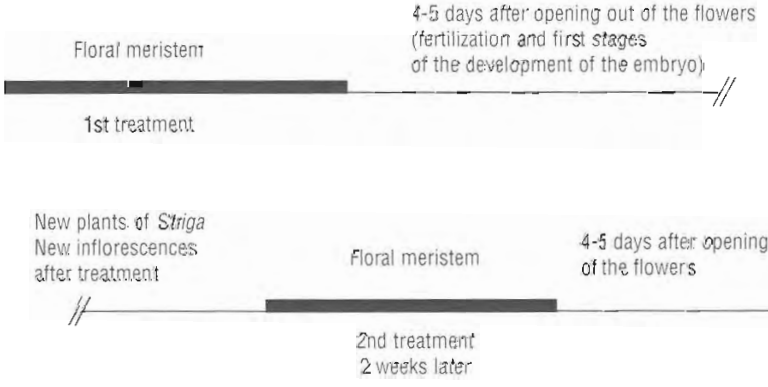

Figure 1

Proposal for the treatment with herbicide in relation to embryological observations (after Paré, 1994).

Introduction

Assuming a vertical gradation in *Striga* seed density (Robinson and Kust, 1962), the distribution of root length density (RLD) in the soil will affect the number of *Striga* attachments that are formed. If *Striga* seeds are predominantly present in surface soil, there is a potential to reduce attachments by means of a root system that forms a smaller proportion of its total root length in the top soil. In contrast to other proposed means of host resistance, such an escape mechanism is based on exploiting the environmentally determined distribution of *Striga* seed in the soil, rather than physiology of *Striga* or host, and is therefore not open to compromise by natural selection operating on *Striga*.

A few studies (Baltus *et al.*,1994; Cherif-Ari *et al.*,1990) indicate that differences in host root characteristics may confer avoidance of *Striga* attachment. However, these studies did not attempt to consider all of the root characteristics that may be important in the avoidance of *Striga*. RLD in any given layer of the soil depends on 1. the proportion of resources allocated to roots or shoots (root fraction) 2. the thickness of roots (specific root length) 3. the pattern of deployment of roots along the length of a root system (root architecture). The picture is further complicated in the case of maize and sorghum in that the root system produced from the seed (seminal root system) is later augmented by nodal roots which develop from further up the stem. Establishing the degree of variation between cultivars in each of these characteristics is a prerequisite to any selection of cultivars which may avoid *Striga* attachment. Furthermore, if selection can be made from cultivars grown under controlled conditions screening would be expedited.

In this study we investigate 1) whether plants of four sorghum cultivars, grown in a uniform environment and supplied with three widely varying levels of nitrogen, differ substantially and consistently in total root length and root system architecture or not, 2) to what extent differences in root architecture observed under controlled conditions are reflected in field- grown plants, 3) whether probability of attachment is related to root length and how the number of attachments per m root length differs among cultivars.

Materials and Methods

1. Growth chamber experiment & growing conditions:

Germinated seeds of four sorghum cultivars (Ochuti, Seredo, Serena and SRN-39) were planted in a cylinder of sand (height:40 cm, diameter:12 cm). Each plant was given daily 100 ml of a 0.4 x Long Ashton solution with 0.33, 1.0 or 3.0 mM nitrogen as ammonium nitrate. Plants received 16 hours light/day, day/night temperatures of 27/20 C and a relative humidity of 75%. The experimental design was factorial (4 cv's x 3 nutrient solutions x 2 repl.). After 17 days roots were carefully separated from the soil, stained, spread on a glass plate and photocopied. Dry weights of roots and shoot were determined.

2. Field trial:

Seeds of sorghum cultivars Ochuti, Serena, SRN-39 and CK-60 were planted at the Kenyan National Sugar Research Station in Kibos, Kenya, in an experimental field with a loamy clay soil. Seventeen days after planting between 8 and 20 plants of each cultivar were harvested with all roots in a block of soil. Photocopies of root systems and dry weights were obtained in a similar way as described above.

3. Relating root length to number of Striga attachments:

Sorghum cultivars Ochuti, Seredo, Serena, and SRN-39 were grown during the summer of 1995

Introduction

The presence of phloem has been convincingly demonstrated in the haustoria of only a few parasites, such as *Phoradendron* (Calvin, 1967) and *Viscum* (Salle, 1976) of Loranthaceae, *Castilleja* (Kuijt and Dobbins, 1971) and *Alectra* (Dorr *et. al.*,1979) of Scrophulariaceae and *Orobanche* (Dorr and Kollmann, 1975) of the Orobanchaceae. The present study demonstrates the occurrence of phloem and the presence of callose deposition on sieve plates in the haustorium of *Scleropyrum wallichianum* an arborescent root hemi-parasite of the Santalaceae for the first time.

Materials and Methods

The haustoria of *S. wallichianum* were collected in and around Perdoor, and Dharmastala of Dakshina Kannada District and Hulical Ghats of Shimoga District in Karnataka State, India. Young and old haustoria, carefully dug out from the soil along with the host roots, were fixed immediately in FAA (40% formalin-glacial acetic acid-70% ethanol).

Standard microtome procedures were followed for dehydration, imbedding and serial sectioning (Johansen, 1940; Sass, 1958). Sections were processed through xylol-ethanol series for staining and mounted in DPX mountant.

Northern's variation of tannic acid-ferric chloride technique with aniline-blue in clove oil as counterstain was employed for routine anatomical observations (Johansen, 1940; Cheadle *et al.*, 1953). Lacmoid-blue was employed as the critical stain for localizing callose on the sieve plates of phloem (Krishnamurthy, 1988). The fluorescence was observed when viewed under Leitz fluorescence microscope using appropriate filters (Currier and Strugger, 1956).

Results

The haustorium of Santalacean root hemi-parasites passes through aunique 'gland' stage during the course of its development (Kuijt, 1977;Niranjana, 1994). In *S. wallichianum* distinct vascular strands are seen extending from the parasite root vasculature to the zone of meristematic cells, above the gland (Fig. 1). These strands consist of both xylem and phloem elements. The phloem includes fully differentiated sieve tube elements, companion cells and phloem parenchyma. The sieve plates are situated on the transverse walls (Fig. 2). Individual sieve elements are recognized by their conspicuous fluorescent sieve plates.

The mature haustorium of *S. wallichianum* is composed of a central parenchymatous region flanked on either side by vascular strands arranged in an arched manner (Fig. 3) (Niranjana and Shivamurthy,1987b). The vascular strands consist of xylem towards the centre and phloem towards the periphery (fig. 3). The phloem includes sieve tube elements, companion cells and phloem parenchyma (Fig. 4). The phloem is observed consistently in both younger and older haustoria at the primary and secondary growth stages respectively.

Simple, and occasionally compound sieve plates are present on the transverse or inclined end walls of sieve tubes. Callose, deposited around the sieve pores responded positively to lacmoid-blue staining (Figs. 2, 4-7). Normally sieve tubes are seen as 1-2 seriate strands in younger haustoria while they are multiseriate in the older ones (Fig. 7). Normal phloem could be traced from the parasite root vasculature through the interrupted zone and the vascular core up to the point where the xylem strands curve for the second time towards the host root (Figs. 3-4).

However, the phloem is not found in the endophyte region.

Discussion

Many of the earlier researchers have reported the absence of phloem in the haustoria of root parasitic Santalales (Kuijt, 1969, 1977). Even when a

the growing bifurcated shoot is active. Figure 3 , longitudinal section of a late-winter shoot, shows this situation. The square in Figure 3 (see arrow) between base of leaf and inflorescence is shown greatly enlarged in Figure 3a. The meristem (see pentagon) is recognizable by its large nuclei. In the following weeks the meristem swells due to increased cell division. This meristem bulge (Fig. 3b) which is typical for other flowering plants, only lasts for a few weeks: differentiation (Figure 3c from May 10th) soon causes this rounded cone to change its shape. The marginal meristem grows more rapidly than the central meristem which causes an indentation. Despite cell division, the centre is inhibited. The shape of the meristem (Figure 3d from June 12th) shows the characteristic inception of the intervention of the flowering impulse. The inhibition in the form of an indentation is the effect of the extremely early flowering impulse. The marginal meristem of the leaf pair distinguishes itself clearly from the tissue of the axis in that it already shows meristemoidal vessels (arrow in Figure 3e from June 27th). All the organs of the bifurcate shoots are recognizable by July. The flower primordium has secondarily developed a conical shape and is surrounded by the two leaf primordium. The synchronous differentiation of the meristem to vegetative and generative organs is a characteristic peculiarity of the mistletoe.

Up to autumn, the axis of the bifurcate shoot has markedly grown and the inflorescence shows a division between the petal and the flower cups. The transverse slit in the flower primordium makes this fact apparent.

The mistletoe stays in this stage of development during the winter growth-pause and one can find a new axillary meristem. On mild winterdays or in the early spring of the following vegetation period, when the mean day-temperature rises above +4¡C, growth starts again. The bifurcate shoot unfolds and after the phase of geotropism it begins its nutation movements. The strictly bisexual flower organs develop further.

The male flower develops until it has pollen grains with two nuclei, and the female flower organs develop a mature embryo sac. This stage is attained in October which is a very early development because the flowering season of the mistletoe is in February (normally this stage of development is typically reached a few days or weeks before flowering). The generative differentiation of the meristem took place 18 months earlier.

After pollination in February/March a whole year will pass before the embryo and fruit are fully ripe.

Discussion

The cycle of development of the mistletoe, i.e., the annual growth of a bifurcate shoot generation from the initial differentiation of the meristem to the ripe fruit, is extremely delayed for the conditions of the humid climate zones.

Even more unusual is the synchronous differentiation of the meristeme into vegetative and generative primordia. The flowering impulse is advanced (acceleration).

The 28-30 day nutation movement period correlates with the flowering impulse. We presume that this nutation movement is endogenous and circalunar. Experiments under constant conditions in climate chambers are in preparation and should give answer to these questions. The diverse directions of these movements make phototropism an unlikely cause. The over-bending theory of Gradmann (1921, which says that a shoot which has come out of its vertical position will react negative-geotropically more than necessary and pass over the perpendicular line in the other direction and thus come into a new swinging movement) can also be excluded because the spherical shape of the mistletoe is an opposite phenomenon. Kunze (1984) also describes bending movements in other plants. However, these plants regain their vertical position when the movements subside. Even fully autonomously

nutating plants reach, with the last growth impulse, their vertical position which can only be explained by an orientation to gravity. The mistletoe distinguishes itself from other plants by the fact that it does not orientat itself by gravity. It acts independently of gravity. Future investigations should adress this problem. The different concentrations of the mistletoe compounds in different organs and at different stages of development were mentioned in the introduction.

Scheer (1992) stresses with respect to the preparation of medicines that "The results showed the necessity of defining harvesting times and recipe of collection organs for the standardisation of mistletoe preparations".

It is clear that a particular stage of development is meant by defined harvesting times (as opposed to a particular date). With this work we have made a step towards being able to define the stages of development of the mistletoe.

Material for histological investigations has been prepared parallel to the harvest for investigations of compounds. We thus have the possibility for a further investigation of the correlation between the mistletoe compounds and stages of development.

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wasps, which are very active during the morning and evening.

Flowers produce abudant nectar. Buds do not secrete nectar, it is exuded once flowers open. Yellow flowers contained significantly greater rewards than the reddish brown flowers. The former contained 30.5 ± 10.2 l of nectar, whereas the latter contained 10.7 ± 2.3 l of nectar (significant at 5% level). The two flowers differed significantly in the percentage of viability of the pollen they contained. The pollen viability of yellow and reddish brown flowers was 95.3 ± 1.2 % and 27.4 ± 3.1 % respectively (significant at 5% level). The two flowers also differed in the stigma receptivity; 85.1 ± 5.2 % of the hand-pollinated yellow flowers on plants set fruit, but none of the reddish brown flowers which were hand pollinated set fruit.

Pollination with or without nectar withdrawal caused change in the petal colour, when compared with the control flowers. The colour change was rapid in the flowers which were outcrossed and nectar withdrawn (Fig-1).Nectar withdrawal affected the colour stage of the flower when compared with the control (Fig-2).

After partial removal of the reddish brown flowers (E) from the experimental plants the arrival of the pollinators to the experimental and control plants was almost similar (Fig-3). However after the complete removal of the reddish brown flowers (E_1) from the experimental plants, they recieved significantly fewer pollinator arrivals than the control (Fig-3).

The changes in chlorophyll, carotenoid and anthocyanin pigments are expressed in Fig-4. Nectar is hexose dominant. Mean amount of sucrose, fructose and glucose of the nectar before and after pollination is given in Table 1. Sucrose is present in small amounts, glucose and fructose in relatively large amounts. The various carbohydrates which were identified in the nectar samples by Thin layer chromatography are given in Table 2.The various amino acid complements identified and quantified in the nectar by TLC and HPLC is given in Table 3.

Discussion

Changes in the flower character of *Santalum album* is manifested by changes in petal colour. The change in petal colour apparently occurs through an increase in the anthocyanin content and a decrease in the chlorophyll and carotenoid content. Selection favouring floral changes occurs when plants benefit from retaining petals on flowers that are unrewarding and that have reduced reproductive potential (Gori,1989). Due to the retention of the petals (in a changed colour) in the flowers the visibility of the plants to pollinators may be increased. Richards (1986) suggested that yellow flowers on a green background of foliage could be less visible to insects. This is clearly manifested in the reddish flower removal experiment, in which the pollinators visitation considerably decreased with the removal of the reddish brown flowers (Fig-3).Eventhough the plants had more rewarding flowers, the pollinators were almost unable to distingush them in the green background. Thus it is evident that the retention of petals (in a changed colour) on spent/unrewarding flowers increases the attractiveness of the plant to pollinators, which in turn enhances the proportion of the flower population that is pollinated, improving seed set (Casper and La Pine, 1984; Gori, 1983, 1989; Lamount, 1985). The petal colour change may be due to pollination,nectar withdrawal, senescence or a combination of these. Our results indicate that the petal colour change is induced due to pollination,nectar withdrawal or by both (Figs 1 and 2). This is supported by the findings of Eisikowitch and Lazar (1987) in *Oenothera drummondii*. The first can be regarded as "pollination induced"(Gori,1983), and the second can also be considered as pollination induced, since pollination is interconnected with nectar withdrawal (Eisikowitch and Lazar, 1987).

Senescence (manifested by the control flowers in the experiment) is experienced by rewardless

flowers (flowers with reduced pollen viability,stigma receptivity and less nectar), when it is associated with change in the petal colour, which can play an important role in directing pollinators towards a more promising reward. This association is advantageous for both flowers and their pollinators (Eisikowitch and Lazar, 1987).

Santalum album comprises of several blooming flowers per plant at the same time. All those yellow flowers on the dark background of the reddish brown flowers can easily be defined as signals anouncing "full reward, ready for a visit". This method of signalling improves the efficiency of visitation of pollinators to suitable flowers (Cruden and Hermann Parker, 1979; Otte, 1974). Therefore this method of signalling may be important both to plant and pollinators.

Nectar is a very important floral reward secreted by floral nectaries. The amount of the reward presented to a flower visitor is an important factor which determines the behaviour of the visitor (Heinrich and Raven,1972).The amount of reward offered to a flower visitor also depends on the concentration of sugar in the nectar. This varies considerably from species to species and shows a clear relation with the predominant pollinator. Since sandal is pollinated by butterflies, bees and wasps, the nectar is hexose rich which coincides with the characteristics of shorttube bee and butterfly flowers as explained by Ananthakrishnan and Raman (1993).

Amino acids are always present in nectars, although in small amounts when compared with sugars. The amino acids occur in amounts that may be significant nutritionally to the pollinator either in protein building or as gustatory stimuli (Baker and Baker,1973 a,b; 1975).Apart from their potential nutrition function, nectar amino acids, with their differing presences, proportions and concentrations can modify the taste of nectars that contain them. So in combination with the sugars, the amino acids may contribute in determining the taste of the nectar to a discriminating flower visitor. Since *Santalum album* is mainly pollinated by butterflies, bees and wasps which depend on the amino acids in the nectar for protein-building (Baker 1975), it contains relatively high amount of amino acids.

One of the important observations pertained to the difference in the quality and quantity of the amino acid / sugar constitution of the nectar produced before and after pollination. The most important among these is the presence of arabinose in the post- pollination nectar and its absence in the pre-pollination stage. Similarly, galactose, although found in both stages, is significantly present in the post-pollination stage. Our field studies have revealed that *Santalum album* has a gametophytic incompatability system where the incompatible pollen tubes are arrested in the style. Previous studies have indicated that in species with gametophytic incompatability the style produces arabinogalactan-rich-proteins (AGPs) and these have been implicated in the control of incompatability systems (Fincher et al. 1983). Probably in *Santalum album* arabinose and galactose are also involved in the formation of stylar arabinogalactan- proteins to have an effective gametophytic incompatability system.

Introduction

According to Teryokhin and Anisimova (1980), species or populations of *Orobanche* parasitizing perennial wild species of host plants are perennial achlorophyllous parasitic herbs too. Weed populations of this species are annual herbs. These authors have fo-und two modes (types) of vegetative propagation in the genus *Orobanche*: "the peren-nial haustorial root type" (in most species of Orobanche) and "perennial tuber type" (in *O. Kotschyi*, related species and some others).

The mode of vegetative propagation in weed populations of *O. cernua* parasitizing sunflower in Europe is a reduced form of "perennial haustorial root type" with very small potential for a multiplication of shoots in tissues of the tubercle. Their secondary haustorial roots ("secondary roots", after Kuijt, 1969; "crown roots" after Kuijt, 1969 and Dorr et al., 1994) possibly can not form secondary haustorial contacts with roots of host plant and produce additional shoots. Wild hosts of *O. cernua* are species from the genus *Artemisia* (*A. maritima* and others) and species from the genera Lactuca, *Xanthium* etc. of the family Asteraceae. (Beck-Managetta, 1930; Tzvelev, 1981). Basic host plants are perennial *Artemisia* spp. *O. cernua* usually attacks *Helianthus annuus* in Europe and *Nicotiana tabacum, Lycopersicum esculentum* and other annual solanaceous plants in Asia.

We have studied the population of *O. cernua* in India that attacks tobacco. Plants from this population have the original mode of development and vegetative propagation.

Materials and Methods

The plant material (seeds, fixed and dry plants) was collected at the experimental farm of Central Tobacco Research Institute (Rajahmundry, Andhra Pradesh, India). Seedlings were grown in the greenhouse of the Komarov Botanical Institute on tobacco by the modified method of Kadry and Tewfic (1956).

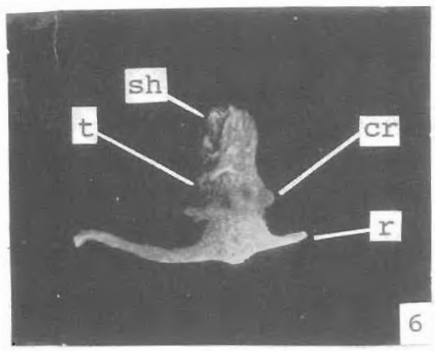
Results And Discussion

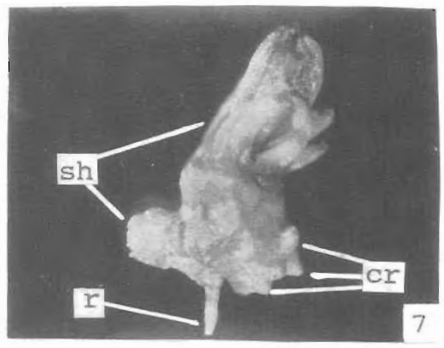
A seedling's development of *O. cernua* on sunflower (Teryokhin and Anisimova, 1978) and tobacco is very similar at the first stages: the penetration of germ-tube (tube - like stage of seedlings) in the tissues root of the host plant, the formation of a tubercle at the place of invasion (Fig. 1-3'). Following are the endogenous or exogenous produc-tion of the main shoot and endogenous production of 1-3 additional shoots from the tubercle as well as a girdle of crown roots (Fig.3,3'). We can see strong differences in the development of seedlings at this stage. A vegetative multiplication of *O. cernua* on sunflower is limited to formation of 1-3 additional shoots from the tubercle. A tubercle is transformed into the base of the main stem (or stems) with a system of crown roots (Fig.3,3',4). A tubercle of *O. cernua* parasitizing tobacco developes in annual tuber with many additional reproductive shoots (up to 50, C.A. Raju personal communication) (Fig.5). The point of a parasitic invasion in root of host plant strong extends (Fig.6,9). Tissues of a parasit and root of host plant are mixed in this region (Fig.9). What is why, an annual tuber of *O. cernua* is some like perennial tubers of *Conopholis*.The first crown roots are situated at the girdle, following roots arise in different places in the tuber (Fig.7-9). New shoots can form between crown roots and above or below others (Fig.8-10).

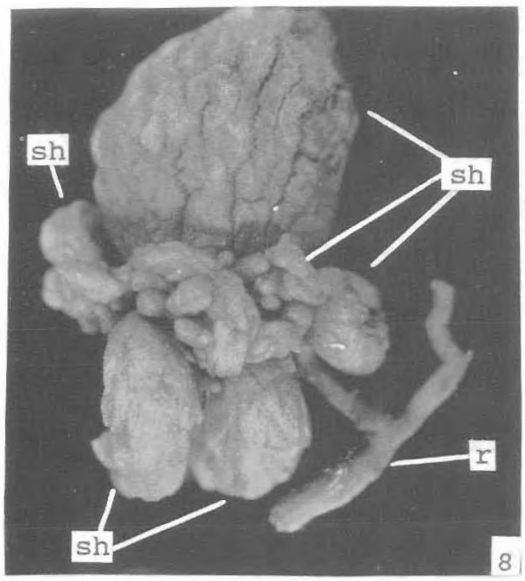
Crown roots (branched and with thick basal portion) loss their function of secondary haustorial contacts, but have reproductive functions. New shoots can arise from basal or lateral parts of crown roots (Fig.11,12). We have named this mode of vegetative propagation the "annual tuber type" (Fig.5).

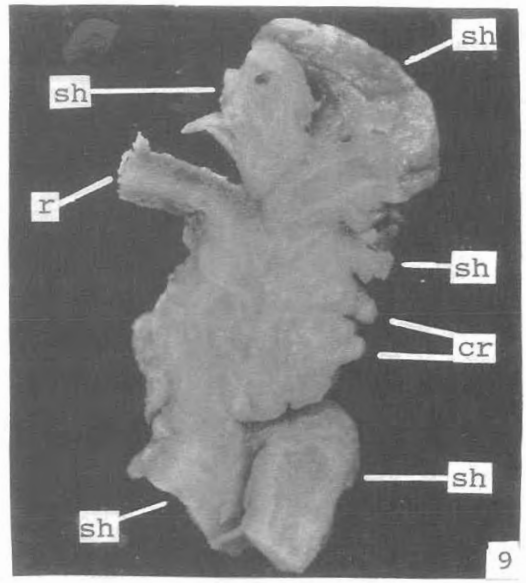
Conclusions

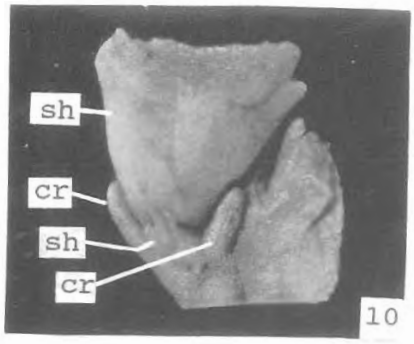
Orobanche cernua parasitizing tobacco in India has the original mode of development as well as vegetative propagation. It is, in our view, the adaptation to long vegetation and peculiarities of

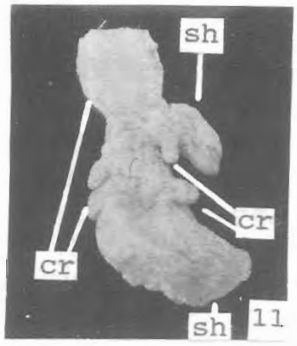


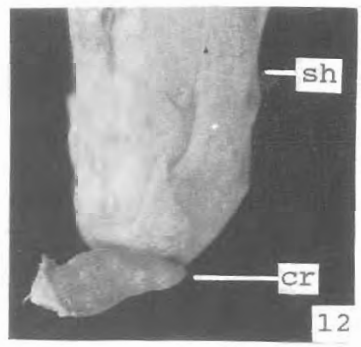












Figures 6-12

Development of a tuber and a vegetative propagation in *Orobanche cernua* ssp. *rajahmundrica*.
Fig. 6 - primary stage development of tuber from tubercle; Fig. 7 - young tuber with reproductive shoots of different age; Fig. 8 - tuber (view from below) with many shoots and young crown roots; Fig. 9 - cut of tuber with shoots and young crown roots; Fig 10 - part of tuber; Fig. 11 - crown root with shoots and new crown roots; Fig. 12 - shoot on lateral side of crown root; (sh - shoot; cr - crown root; r - root of host - plant; t - tubercle.

III.9

TYPES AND FORMS OF VEGETATIVE PROPAGATION IN THE OROBANCHACEAE AS A RESULT OF DIFFERENT ADAPTIVE STRATEGIES

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ABSTRACT

We have found a new type of vegetative propagation in weed populations of *Phelipanche spp.*(= *Orobanche spp.*), namely *P. aegyptiaca*, *P. mutelii* and *P. ramosa*: the annual bulb-like type. Thus there are four types of vegetative propagation in the Orobanchaceae , i.e. two perennial types and two annual types. Adaptive peculiarities of these types are discussed in connection with the modes of life of host plants.

Key words: *Phelipanche, Orobanche*, evolution, tubercle, tuber.

really new shoots (not branches), because in the base of these new shoots, new "bulb-like" structures were formed, sometimes with a new crown root system. The presence of scales on the "bulb-like" structure confirm its stem nature (Fig. 4). In the "bulb-like" structures new shoots can be formed only above the zone of crown roots.

In the genus *Phelipanche* the basic mode of vegetative propagation in wild populations is the branching of stems and development of a secondary haustorial root system. This was the first mode of vegetative propagation in the evolution of *Phelipanche* species. We consider the formation of new (daughter) shoots in bulb-like structure in plants from weed populations in *Phelipanche spp.* as a new type of vegetative propagation of broomrapes: the annual bulb-like type. The new shoots from bulb-like structures are formed during one vegetative season.

Thus, including the data of Teryokhin and Anisimova (1980) and Teryokhin et al. (this volume), there are four types of vegetative propagation in the Orobanchaceae family:

I.- perennial haustorial root type: in wild species of *Orobanche* and *Phelipanche* (Fig. 1a, normal form) and in *Phacellanthus tubiflorus* (Fig. 1b, extreme form);

II.- perennial tuber type: *Boschniakia rossica* etc. (Fig. 2);

III.- annual tuber type: in some weed populations of *O. cernua* which parasitises tobacco (Fig. 3);

IV.-annual bulb-like type: in some weed populations of *Phelipanche spp.*(Fig. 4).

We can see from this scheme that the perennial haustorial root type (Fig. 1a, 1b and 1c) has three forms. The reduced form (Fig. 1c, 1c') is represented in weed populations of *Orobanche cernua* which parasitise sunflower in Europe. In these populations an annual vegetative propagation is achieved by endogenous or exogenous formation of apices of new shoots from tissue of tubercle only (Fig. 1c'). The system of crown roots is reduced and probably does not form new shoots. The "normal", most widespread form (Fig. 1a) of "perennial haustorial root" type is, in our opinion, the ancestral form for all other secondary adaptive types and forms of vegetative propagation in the family Orobanchaceae (see scheme).

The analysis concerning the variety of host plants reveals that different types and forms of vegetative propagation are connected with the life of the host plants. The ancestral form ("normal" form of "perennial haustorial root" type) is connected with wild perennial species of host plants, especially with perennial herbs. An extreme form of this type (*Phacellanthus* form) is associated with trees (e.g. *Fraxinus spp.*) which have a dense net of roots. The "perennial tuber" type is characteristic for wild species of broomrapes which parasitise host plants with a loose net of roots. For example *Orobanche* species which parasitise taxa of the Umbelliferae, especially in arid and subarid regions, have this type.

The formation of annual adaptive types of vegetative propagation (annual tuber type and annual "bulb-like" type) was brought about by changes in reproductive strategies of *Orobanche* and *Phelipanche* species in connection with the transition of some populations from "wild" perennial to the "weedy" annual mode of live. As perennials, it is necessary to produce new shoots every year. For annuals, there is a necessity to produce shoots in the same season, i.e., intensify the seed reproduction.

Teryokhin et al. (this volume) concluded that the time for evolutionary development of the "annual tuber" type is ranging from 300 up to 10 000 years, for the reduced form of "perennial haustorial root" type not more than 300 years. The historical time for development of the "annual bulb-like" type in weed populations of Phelipanche spp. can not exceed the age of agricultural history. This is very fast for the evolution of new morphological adaptations.

Conclusions

A new type of vegetative propagation (annual "bulb-like" type) is described in weed populations of *Phelipanche* (= *Orobanche*) species.

In the Orobanchaceae there are four types of adaptations for vegetative propagation, today.

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Introduction

Studies of the seeds of the semi-arid Mediterranean species Cuscuta pedicellata Ledeb. which mainly parasitises leguminous plants showed interesting adaptive structures. Seed morphology changes between pitted and papillous appearances depending on whether the seeds are dry or moist, respectively. Based on this, it was suggested that the dry pitted seeds are adapted to wind dispersal, the papillous appearance to efficient water uptake prior to germination (Lyshede, 1984). Knepper et al. (1990) found it possible to identify seeds of the three Cuscuta subgenera using size, shape, hilum type and position on dry seeds. The seed coat consists of an epidermis, two different palisade layers, and a multiple layer of compressed parenchyma cells. The outer palisade cells develop an additional inner wall layer, thus resembling the U-shaped palisade cells of many cruciferous seed coats (Lyshede, 1984, 1992). The inner palisade layer is similar to the Malpighian cells in leguminous seeds, in which it forms the seed coat epidermis. The embryo is embedded in the endosperm which has a rather thick cuticle on its outer cell walls and further comprises an aleurone layer surrounded by cellulose-pectinaceous cell walls, some rows of starch cells, and thick masses of hemicellulosic cell walls. The endosperm cell walls become mucilaginous and swell in imbibed seeds (Lyshede, 1992). In C. campestris Yunck. the endosperm is absorbed during seed development. Due to the similarity of the endosperm in C. pedicellata to that of some leguminous seeds with galactomannan cell walls (Reid, 1971), its polysaccharide composition was studied. The results hereof are brought in this report together with further structural investigations of the seed coat, especially the hilum. Seed studies of the temperate species C. europaea L. was carried out for comparison.

Materials and Methods

Light Microscopy (LM)

Water-imbibed seeds of *C. pedicellata* and *C. europaea* were opened by a slit or cut in halves. Following fixation in FAA and dehydration in an alcohol series the seeds were embedded in Technovit 7100, sectioned, and stained with various histochemical stains to detect lipids, proteins and polysaccharides.

Scanning Electron Microscopy (Sem)

Water-imbibed seeds of the two species were cut in halves. Some were fixed in 3% glutaraldehyde, dehydrated in an acetone series prior to critical point drying and gold sputtering. Some were cryo-fixed in liquid nitrogen and transferred to a cryo-stage in a JEOL 840A SEM for examination. Dry seeds were only gold sputtered before investigation.

Transmission Electron Microscopy (Tem)

Water-imbibed seeds of *C. pedicellata* were fixed in 3% glutaraldehyde and 1% OsO4 in 0.1 M phosphate buffer. After dehydration and embedding in Epon ultrathin sections were stained in uranyl acetate and lead citrate before examination in a JEOL 1200 EX transmission electron microscope.

Carbohydrates in Cuscuta pedicellata endosperm cell walls. Batches of twenty seeds were soaked in water over night and the endosperm separated from the seed coat and embryo by hand under a dissecting microscope. The endosperm was treated with hot 70% methanol (MeOH) to remove soluble oligosaccharides followed by enzyme treatment (salivary amylase and pure endo-(14)-ß-D-mannanase, respectively), hydrolyzation with trifluoro acetic acid (TFA), and analysis by injection into a Dionex HPAE comparing with a standard sugar solution and by thin layer chromatography. A hot water extract was purified by copper-precipitation, TFA hydrolyzed and analyzed for polysaccharides.

Results

Microscopy. The structure of the seeds of *C. pedicellata* and *C. campestris* exclusive of the hilum has been described earlier using light- and electron microscopy (Lyshede 1984, 1992). Fig. 1 shows the embryo surrounded by endosperm and seed coat. The outer palisade layer stains reddish with Sudan IV suggesting suberization of the cell walls. The inner palisades have a "light line" which stains a bright red colour with PAS/Anilin-Blue-Black (PAS/ABB) (Fig. 2) and the cell walls stain partly red, partly blue showing a mixture of polysaccharides and protein. Staining with safranin gives no lignin reaction in these walls. In polarized light the "linea lucida" shows birefringence when positioned at an angle of about 45 with two crossed polarization filters. In the SEM a bulge is seen in the palisade cell walls close to their distal part making a tight attachment between neighbouring cells (Fig. 3). The distance between the bulges and the outer border of the palisade cell walls is the same as for the light line measured in LM. Regularly, lens shaped spaces the length of the cells are seen in the inner palisade layer by LM and SEM. Although they may be artifacts caused by stress or release of stress during the preparation, it is believed that such intercellular spaces occur in the seed coat. The seed coat of *C. europaea* is in general similar to that of *C. pedicellata*. The epidermal cells are smaller, but become papillous when moist as in *C. pedicellata*, (Fig. 1). The cell walls of the inner palisades stain a more intensive red with PAS/ABB with no blue reaction. In the hilar area of both species the cells of the innermost palisade layer become longer than those of the seed coat itself, each subsequently dividing into two or more cells by periclinal divisions. The outer of these cells remain palisade-like while the inner ones are more or less isodiametrical (Fig. 4 & 5). The U-shaped palisade cells become narrow and thick-walled in the hilum and they are not suberized. In contrast to the cells of the seed coat proper they stain red with PAS/ABB. The outer part of the cells tapers toward the epidermis that likewise narrows with the cells tapering inwards toward the palisade cells. Thus a very tight connection is formed between these two cell layers. In the SEM the hilar characteristics of thick walled palisade cells are confirmed for both species (Fig. 6).

Endosperm. Whereas the endosperm in *C. pedicellata* is easily removed from the seed coat in imbibed seeds, this is not the case in *C. europaea*. Otherwise, the endosperm of the two species appears quite similar. Both have a cuticle and the walls surrounding the aleurone cells different from the mucilaginous thick hemicellulosic walls internal to the aleurone layer. Starch cells seem, however, to be present throughout the endosperm in *C. europaea* and the mucilaginous walls appear "fibrous" in contrast to *C. pedicellata*.

Carbohydrates in endosperm cell walls. The extraction procedures and the analysis by HPAE and TLC show that the endosperm cell walls in *C. pedicellata* consist of a galactoglucomannan composed of 7.6% galactose, 9.8% glucose, and 82.6% mannose. The composition of the endosperm cell walls has not been investigated in *C. europaea*.

The embryo contains proteins in both species. In *C. pedicellata* the cells also contain lipids which occur very scanty in *C. europaea*. The embryo epidermal cells were reinvestigated in the TEM to see if transfer cells would play a role with uptake of metabolites released from the endosperm, but no specialized cell walls were detected.

Discussion

Seeds of *Cuscuta pedicellata* have earlier been described in detail by light and electron microscopy (Lyshede 1984, 1992). The inner of the two palisade layers is of special interest due to its similarity to the epidermal Malpighian cell layer in most leguminous seed coats. Like these, the inner palisade cells of *Cuscuta* comprise a light line. A change of microfibrillar direction in *C. pedicellata* was seen in the TEM and suggested to be the cause of the light line (Lyshede 1992). Other authors believe that the light line (in Leguminosae) is the

border between different chemical substances in the cell walls (e.g. Harris 1983). The localized bulging in the palisade cell walls, seen in cut seeds of *C. europaea*, is at the same measured distance from their outer part as the light line seen in the LM. A similar bulging was also noticed in the palisade cell walls in the seed coat of *Trifolium repens* L. in which additional foldings of the distal part of the cell walls were observed (Martens et al. 1995). The bulges make a tight interconnection between the palisade cells. Most species of *Cuscuta* have dormant seeds. The light line may play a role as one reason for dormancy in *Cuscuta* as it may in leguminous seeds. Another cause may be the outer suberized palisade layer. Dormancy may depend on whether the large intercellular spaces of the same length as the palisade cells pass the light line in intact seeds. The intercellular spaces may otherwise function in efficient water uptake.

In the hilum, the seed coat structure changes abruptly. The inner palisade cells become longer, more thick-walled and divide periclinally in their inner part. The outer palisade cells change completely from cells with a large lumen to dense thick-walled cells, each one tapering into the disc of narrow epidermal cells. The differences in stainability with Sudan IV, PAS/ABB, and Safranin from the non-hilar cells are noteworthy. There are some similarities between seeds of the Leguminosae and *Cuscuta* regarding cell types and specialized hilar areas. In leguminous seeds, Hyde (1954) described the valve-like function of the hilum responsible for the drying of post-ripening seeds. During lower relative humidity in the surroundings the hilum opens due to the differences in cell wall composition between the palisade and counter-palisade layers and moisture is able to escape from the seeds. With higher relative humidity in the surroundings the hilum closes and moisture cannot enter the seed. Whether a similar mechanism is present in Cuscuta cannot be told from structure alone, but it seems a possibility. The endosperm galactoglucomannan cell walls in *C. pedicellata* become mucilaginous and swell during imbibition. This ensures a good contact with the embryo during initial germination as seen by imprints of the embryo. The water does not easily evaporate due to binding forces and the cuticle. This is of importance under semi-arid conditions where drought may follow rainy periods and it ensures that germination is not halted and that the seedling keep alive. This was also suggested by Reid (1971) for the legume *Trigonella foenum-graecum* L. which has a galactomannan endosperm with similar properties. The temperate *C. europaea* is also similar, but may have an endosperm of different composition as seen by its "fibrillar" appearance. *Cuscuta campestris* without endosperm in mature seeds may have a different germination strategy. The endosperm is completely digested during germination. The embryo cells do not, however, contain structural features such as transfer cells as an indication of metabolite uptake. This is a feature of the embryo in other plant species: *Trifolium repens* (Jakobsen et al. 1994) and *Vicia faba* L. (Johansson and Walles, 1993) which, however, both digest the endosperm during seed development.

The seeds of *Cuscuta* contain a food package consisting of proteins and lipids in the aleurone cells, starch, and a mucilaginous hemicellulose in the endosperm. In addition, the embryo contains protein and often lipids, but little starch. This ensures a reasonable start of the hazardous life of these plants.

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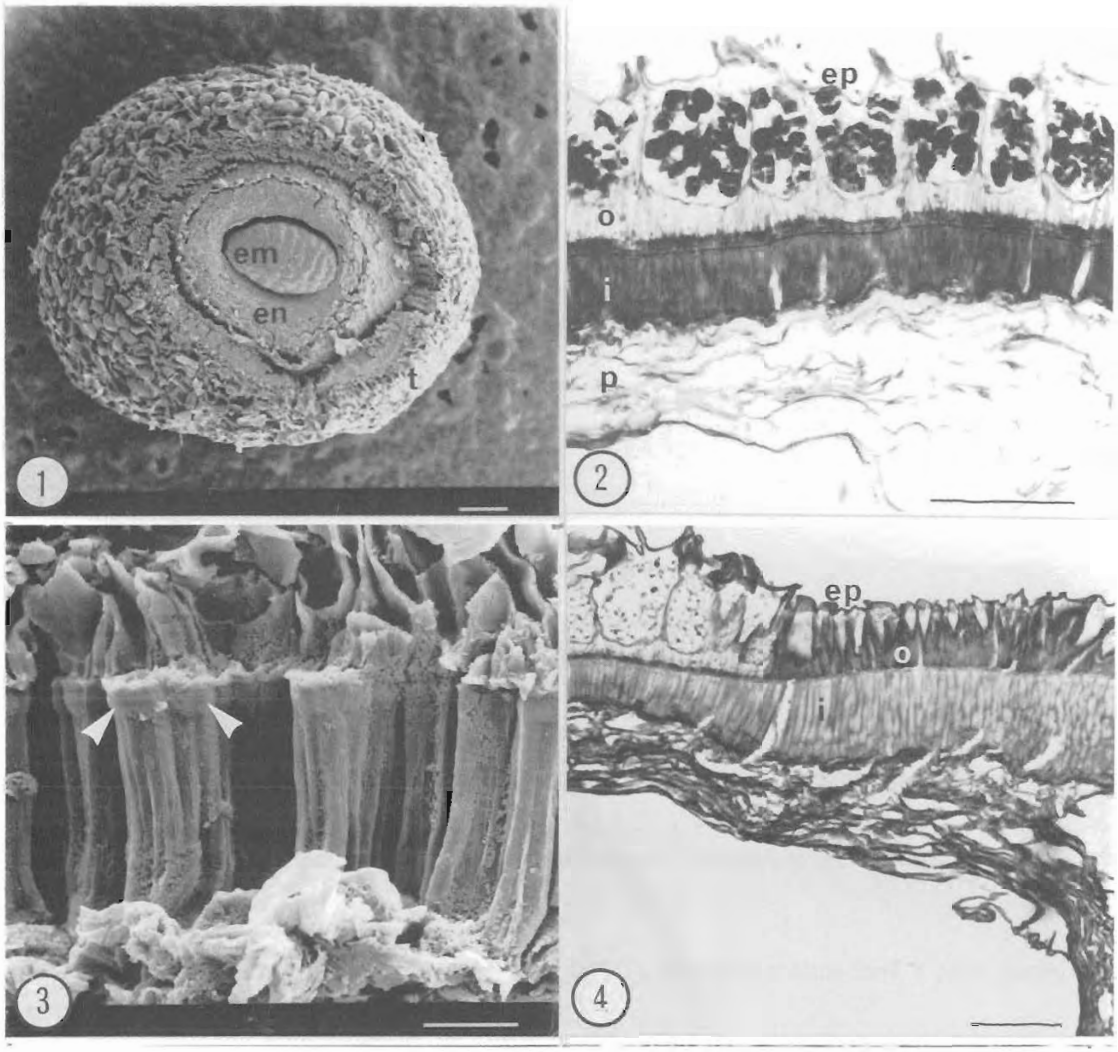


Figures 1-4

1. *Cuscuta europaea*. Chipped seed showing embryo (em), endosperm (*en*), and seed coat (testa (t)). SEM. Bar: 100 m.
2. *Cuscuta pedicellata*. Section of seed coat showing epidermis (ep), outer palisade (o), inner palisade (i), and parenchyma (p). Note the densely stained "light line" in inner palisades. LM stained with PAS/ABB. Bar: 50 m.
3. *Cuscuta europaea*. Inner palisade cells with bulges on distal part of cell walls (arrowheads). SEM. Bar: 10 m.
4. *Cuscuta pedicellata*. Section of hilum showing thick walled outer palisades (o) in close connection with epidermis (ep). LM stained with Toluidine Blue. Bar: 50 m.

Introduction

Plant trichomes have interested botanical scientists for a long time. During the last two centuries, the use of trichomes in taxonomic delimitations has been stressed by many workers (Solereder, 1899, 1908; Metcalfe and Chalk, 1950; Hummel and Staesche, 1962; Uphof, 1962) and since the widespread use of SEM techniques, trichomes have been used more and more in the classification of genera and species (Rivera-Núñez and Obón-de-Castro, 1992).

Trichome studies in the Scrophulariaceae mostly concern glandular trichomes of European taxa in the parasitic subfamily Rhinanthoideae (Fedorowicz, 1916; Weber, 1975; Bolliger, 1985). Recently, Raman (1987, 1991) studied trichomes on the corollas of 71 genera and 193 species to establish taxonomic relationships within the Scrophulariaceae and related families.

We examined the indumentum of eight African taxa of the Buchnereae tribe belonging to the genera *Striga, Buchnera* and *Rhamphicarpa.* As the subgeneric relationships within the genus *Striga* are not well understood (Musselman, 1987), one goal of this study was to look for taxonomically useful characters. Another purpose was to learn if a higher degree of indumentum specialization can be found in plants belonging to related genera, and showing a more advanced evolution. Trichomes were investigated in plants of six *Striga* species, including the most important food crop parasites *Striga hermonthica* (Del.) Benth. and *Striga gesnerioides* (Willd.) Vatke, as well as four other species, *Striga* aspera (Willd.) Benth., *Striga brachycalyx* Skan in Dyer, *Striga klingii* (Engl.) Skan in Dyer and *Striga macrantha* (Benth.) Benth., which, up to now, are considered as parasites of wild grasses (Raynal-Roques, 1994). Two other parasites, *Buchnera hispida* Buch.-Ham. ex D. Don and *Rhamphicarpa fistulosa* (Hochst.) Benth., reported as crop parasites (Sallé et al., 1994), are included. It is noteworthy that these species only develop secondary haustoria, in contrast to *Striga* which develop primary and secondary haustoria.

Finally, an important motivation for this study is the conviction, that a better knowledge of the taxonomy and biology of parasites should lead to better control (Sallé et al., 1995).

Materials and Methods

Plant Material

For the different species, trichomes of all aerial parts were investigated except the gynoecium and androecium. The material was collected either from natural sites, where it was immediately fixed in FAA, or grown under greenhouse conditions.

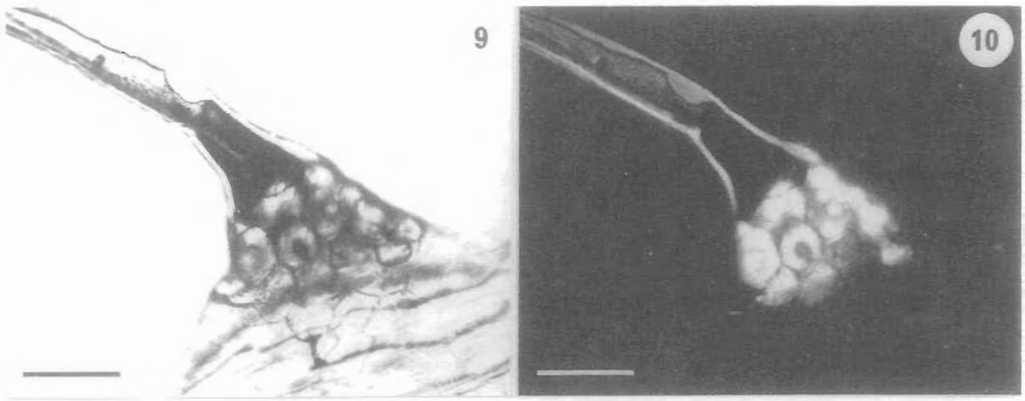
Scanning electron microscopy (SEM)

Samples were fixed with 4 % glutaraldehyde in 0,1 M sodium cacodylate buffer (pH 7,4) for 1 h at room temperature. The specimens fixed in FAA were also treated in this manner to obtain completely turgescent cells. After washing in buffer, the material was dehydrated in a graded ethanol series, critical point dried with CO_2, mounted on stubs using double-sided sticky tape and coated with a thin layer of gold. Observations were carried out on a JEOL JSM-840 and a Philips SEM 505 scanning electron microscope.

Results

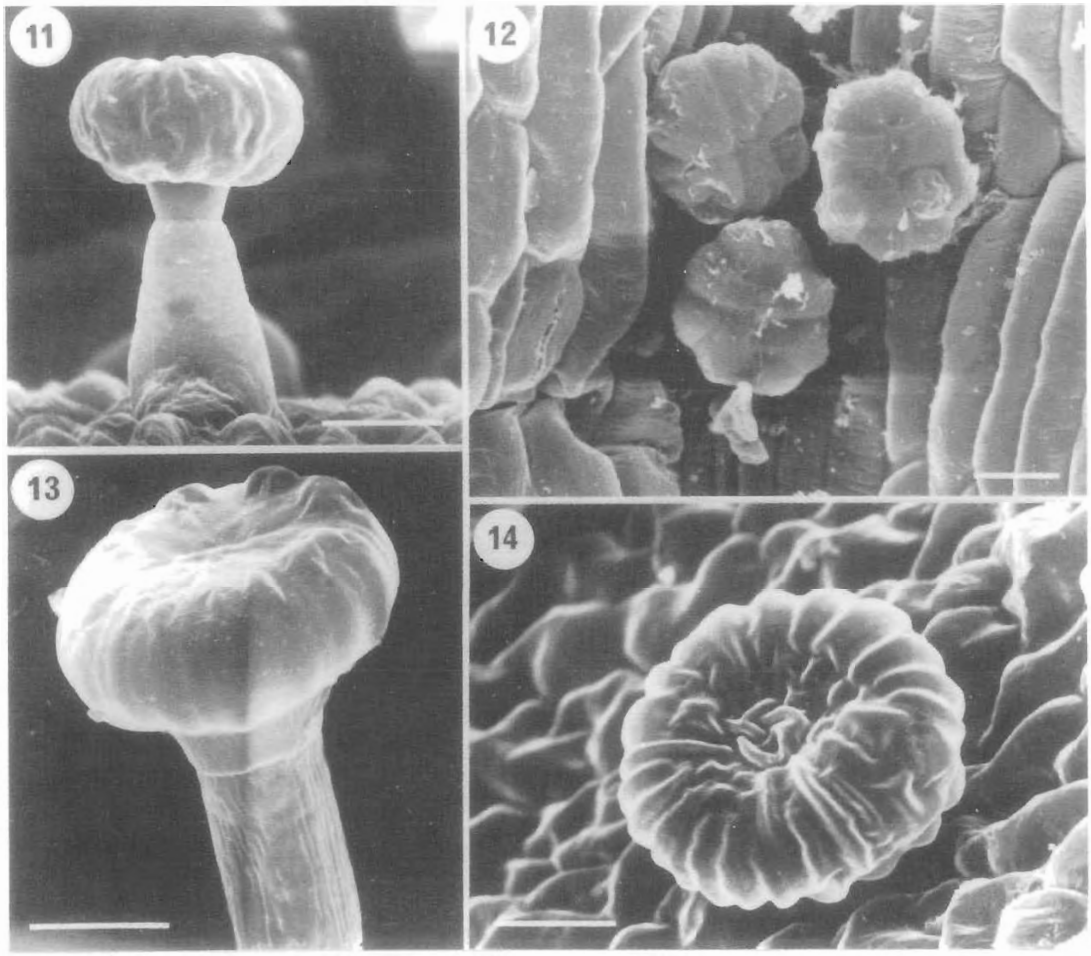
Trichome types

In the investigated species, the indumentum can be divided into two groups. The first one is a large group of ubiquitous glandular and nonglandular trichomes observed on many aerial organs of the different species and has no taxonomic significance. It has not been described here. The second includes several characteristic trichome types that are restricted either to chlorophyllous organs or to the corolla of one or at least a limited number of species. The latter will be described in



Figures 9-10

Mineral deposits in nonglandular trichomes on the adaxial leaf surface of *Striga hermonthica*. Bars: 30 µm. Fig. 9: normal light. Fig. 10: polarized light.



Figures 11-14

Characteristic glandular trichomes. Bars: 20 m. Fig. 11: *Rhamphicarpa fistulosa*, leaf, adaxial surface. Fig. 12: *R. fistulosa*, leaf, adaxial surface. Fig. 13: *Striga macrantha*, stem. Fig. 14: *R. fistulosa*, calyx, abaxial surface.

III.12

THE EFFECT OF NITROGEN ON *Orobanche* AND *Striga* - STATE OF THE ART

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ABSTRACT

Recent literature on the effects of nitrogen on *Orobanche* and *Striga* is reviewed and discussed in relation to previously published research. In line with results of earlier studies on various *Orobanche* and *Striga* species, it was confirmed that ammonium-N directly inhibits *in vitro* seed germination in *Orobanche crenata*. In addition it was shown that ammonium-N inhibits attachment of *Striga hermonthica* radicles to host roots and development of *S. hermonthica* shoots. However, it appeared that the effect of ammonium-N in the form of ammonium nitrate is different from the effect of other ammonium compounds. Ammonium nitrate did not directly affect germination of *S. hermonthica* seeds, which seems in accordance with an earlier report on *Orobanche ramosa*. Moreover, in contrast to ammonium sulphate and ammonium chloride, ammonium nitrate did not inhibit shoot development of *S. hermonthica* on a nutrient medium in the absence of a host. It was concluded that the effect of NH4+/NO3- ratios on the development of these parasitic weeds merits further study. In addition, it was confirmed that N-fertilizer inhibits stimulant production in sorghum. In field studies it was again shown that application of nitrogen fertilizer may reduce infestation by *Orobanche* and *Striga*.

Additional key words: broomrape, witchweed, faba bean, cereals

inhibition of germination of *O. crenata* seeds was concerned. In van Hezewijk's experiments urea appeared to be totally ineffective when applied during conditioning, in contrast to ammonium sulphate, which strongly inhibited germination, especially in combination with a nitrification inhibitor (N-serve). During the germination phase both ammonium sulphate and urea decreased germination (as well as the length of the radicles). However, the effect of ammonium sulphate was more pronounced. Van Hezewijk (1994) did not test the effect of ammonium nitrate on in vitro seed germination of *Orobanche*.

In addition, van Hezewijk (1994) conducted pot experiments with *O. crenata* on faba bean. She demonstrated that application of urea, ammonium sulphate or ammonium nitrate resulted in 50-60% fewer *O.crenata* attachments per host plant. Potassium nitrate, on the other hand, was ineffective.

Results of field experiments showed that the effect of glyphosate on *O. crenata* parasitizing faba bean is enhanced by adding ammonium sulphate to the spraying solution (Ramírez-Ortega et al., 1992). Unfortunately, phytotoxicity of glyphosate to faba bean was also enhanced by adding ammonium sulphate.

Demirkan and Nemli (1994), working in Turkey, observed that the number of *O. ramosa* shoots in tomato decreased as the level of N (applied in the form of ammonium sulphate + ammonium nitrate) was increased from 0 to 16 kg/ha.

Striga

Earlier work:

Regarding seed germination, ammonium-N brought about a similar decrease in germination and shortening of the radicles in *Striga*, when tested *in vitro*, as in *Orobanche*. This was found for *Striga hermonthica* Benth. (Peschi and Pieterse, 1982; Linke, 1987; Kroschel, 1989; Pieterse, 1991) and *Striga asiatica* (L.) Kuntze (Okonkwo, 1991).

In addition, various authors reported a decreased stimulant production by sorghum as a result of nitrogen application (Teferedegn, 1973; Sherif and Parker, 1986; Kroschel, 1989; Raju et al., 1990). Teferedegn (1973), who tested the effect of potassium nitrate and ammonium nitrate, reported that ammonium nitrate was less effective. Sherif and Parker (1986) found that ammonium sulphate as well as another inorganic nitrogen source, which was not specified, reduced stimulant production in sorghum by about half, whereas chicken manure had no effect. Kroschel (1989) tested the effect of ammonium sulphate, urea and calcium nitrate. These compounds significantly inhibited stimulant production by sorghum roots. However, the effects of ammonium sulphate and urea were more marked than those of calcium nitrate. Raju et al. (1990) applied ammonium nitrate.

Kroschel (1989) reported that ammonium sulphate and urea inhibit early development of *S. hermonthica* on sorghum roots. This effect was not caused by calcium nitrate.

Recent work:

Cechin and Press (1993a,b,c) and Press and Cechin (1994) conducted detailed studies on the effect of ammonium nitrate on the *Striga hermonthica*-sorghum association. Their studies were conducted in polyethylene bags as well as in pots where they observed that ammonium nitrate indirectly inhibited germination of *S. hermonthica* seeds. This effect was caused by reduced stimulant production, not by a direct influence on seed germination, the germination recognition system or the stability of germination stimulant once exuded from sorghum roots (Cechin and Press, 1993a; Press and Cechin, 1994).

Following germination, ammonium nitrate inhibited subsequent attachment of the parasite and its early

development on host plant roots (Cechin and Press, 1993a,b; Press and Cechin, 1994).

Cechin and Press (1993b) and Press and Cechin (1994) also reported that ammonium nitrate stimulates photosynthetic activity of both parasite and its sorghum host. An increase in the ammonium nitrate supply resulted in a decrease in the inhibiting effect of *Striga* on its host's biomass accumulation. In rice, on the other hand, ammonium nitrate did not bring about a marked alleviation of the effect of *S. hermonthica* (Cechin and Press, 1994). In addition it was found (Cechin and Press, 1993c) that the influence of *S. hermonthica* on sorghum growth depends on the provenance of the parasite seed and also that the effect of nitrogen supply on the relationship between host and parasite differs according to seed provenance.

This year, Igbinnosa et al. (1996) reported the effect of various forms and various rates of nitrogen compounds on shoot development of *S. hermonthica* on a sterile nutrient medium in the absence of a host plant. Increased concentrations of potassium nitrate, sodium nitrate, calcium nitrate and magnesium nitrate led to a significant increase in *S. hermonthica* shoot development. On the other hand, increased concentrations of ammonium sulphate, ammonium phosphate, ammonium chloride and urea significantly reduced *S. hermonthica* shoot development. Ammonium nitrate did not suppress the shoot length and dry weight of *S. hermonthica* plants.

Gworgor and Weber (1991) observed that osmotic pressure in *S. hermonthica* parasitizing sorghum decreases in the presence of N-fertilizer. Consequently, the flux of water and nutrients to the parasite is decreased.

In trials with N-fertilizer in Southwestern Kenya (nitrogen source not specified) and India (nitrogen source was urea) no significant reduction of *Striga* infestation could be observed (Smaling et al., 1991; Osman et al., 1991). On the other hand, more promising results were obtained by Odhiambo and Ransom (1994) and Ransom and Odhiambo (1994). They conducted field experiments in Western Kenya for four successive seasons to determine the long-term effects of stover management, fertility level and hand-weeding on the density and seed numbers of *S. hermonthica* in the soil and on maize yield. Fertilizer applications (the nitrogen source was not specified) substantially reduced *Striga* numbers by the fourth season, particularly if combined with hand-weeding.

Verkleij et al. (1994), working in the Republic of Benin, observed that a single urea treatment 5 days after sowing, more or less irrespective of the concentration, eventually led to a higher number of *Striga* plants compared to the control. On the other hand, when the urea treatment was repeated 21 days after sowing, the average number of *Striga* plants was lower than in the control. It was concluded that the timing of the urea application was more important than the total amount applied.

When a commercially available, liquid formulation of nitrogen fertilizer (containing 174 g ammonium nitrate and 137 g urea per litre) was sprayed on the soil surface at rates higher than or equal to 44 ml/m2, it had an adverse effect on *S. asiatica* seeds at or near the soil surface (Eplee et al., 1994). When this nitrogen fertilizer, together with a surfactant, was applied to *Striga* seed pods, it was effective in killing 98% of the *Striga* seeds. In the absence of a surfactant 64% of the seeds were killed.

General Discussion

The effects of nitrogen on in vitro germination of *Orobanche* and *Striga* have been confirmed in recent studies. For *Orobanche* this is a direct effect, which was previously also observed for *Striga*. For *Striga* seeds in the vicinity of sorghum roots there is also an indirect effect via decreased stimulant production. The direct effect is caused by ammonium-N, not by nitrate-N, whereas the indirect effect is caused by ammonium-N as well as by nitrate-N.

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Introduction

In view of the complex inter-relationships between the crop hosts and the attached root parasites, it has been difficult to study the growth characteristics and requirements of the parasites in order to enable development of effective and sustainable control measures. Aseptic culture of embryos facilities experiments to determine the factors that regulate the growth and development of the organs of the seedling plant. It also aids the study of the biochemistry and metabolism of germination, otherwise difficult to assess in the embryo enclosed within seeds. Culture of isolated parasite seeds, embryos, and seedlings in the absence of the host provides a unique opportunity for assaying various growth regulatory functions in the parasites, and the effects of specific metabolites and substrates on the development of the parasitic plants, and thus infer the extent of their dependence on their hosts. Emphasis in this overview of aseptic culture studies and their implications on the biology and control of parasitic weeds will be focused mainly on *Striga* as it is, by far, the most economically important of the parasitic weeds, and the most intensively studied.

Initial Results, Achievements, and Problems

Development of *Striga* seedlings without attachment of a host was first reported by Worsham *et al.* (1959) who observed that *S. asiatica* seeds treated with cytokinins e.g. kinetin and other 6-substituted aminopurines led to seed germination and elongation of plumule beyond the cotyledons, but with no further development. Subsequently, Williams (1961) reported that light, GA, and kinetin induced morphogenetic changes in *Striga* seedlings, and that certain factors in the host rhizosphere influenced the morphogenesis of the seedlings. Then followed the report of Okonkwo (1964, 1966) on definitive aseptic cultures and achievement of flowering in cultured *Striga hermonthica*, followed by other studies as summarized in Table 1.

Okonkwo's results with *S. hermonthica* (1966), *S. gesnerioides* (1982), and *S. asiatica* (1991), as well as on *Alectra* spp.(1975), and studies by Chidley and Drennan (1987), and by Riopel and Baird (1987) showed that shoot development in these parasites would be dependent on supplies of water, mineral salts and sugar. This would suggest that these are the minimal requirements of the parasites from the host in order to develop a shoot system and achieve normal growth. However, the results of Yoshikawa *et al.* (1978) indicated that *S. asiatica* requires cytokinins, in addition to mineral salts and sugar, to develop a shoot system in vitro, suggesting its host-dependence for mineral salts, sugar, and cytokinins. Cai *et al.* (1993) have attributed these differences in results to differences in seed treatment since Okonkwo (1982, 1991) and Chidley and Drennan (1987) used conditioned *Striga* seeds stimulated to germination by strigol analogues, GR - 24 and GR - 7. On the other hand Yoshikawa *et al.* (1978) tested unconditioned seeds and Cai *et al.* (1993) tested both conditioned and unconditioned seeds, and neither inoculated pregerminated *Striga* seedlings on their culture media.

Okonkwo's (1966) point was determination of the minimal requirement from the host for a germinated parasite seedling to initiate shoot development. The rooted parasite seedling is apparently capable to make hormones such as auxins and cytokinins, and thus would not mandatorily require these hormones to be supplied by the host, after germination. It is known that cytokinins are synthesized by roots in plants. Even the radicles of germinated *Alectra* seedlings have been shown to produce hormones capable of stimulating root initiation in the legume host (Visser, Dorr and Kollmann, 1977; Dor, Visser and Albers, 1977. More recent studies (Cai *et al.*, 1993) indicated that isolated *Striga* cultured root produced cytokinins that stimulated shoot development in the culture. The results of Yoshikawa et al. (1978) and Cai *et al.* (1993) on the role of cytokinin in *Striga* morphogenesis can be interpreted as stimulation of (abnormal) germination, followed, by shoot development

elongation), and so can be applied when crops are present since the germinated seeds are unable to attach to host roots. Besides, recent reports on rapid screening of maize genotypes for low stimulant production, using the "agar gel technique" are encouraging. In this technique, the indices for germination and haustorium formation were simply to measure the furthest distance from the root of maize at which *Striga* seed was found germinating, and forming haustoria initials, respectively (Fasil *et al.*, 1994). Aseptic cultural studies may be used to isolate and screen sorghum or other host strains or cultivars for their production levels of the haustorium development signal. Those cultivars or strains that produce abnormally low amounts of haustorial signals, but high amounts of germination stimulants should have a double advantage of being resistant to *Striga* as well as deplete *Striga* seed population in the soil.

Striga-derived toxin: Some varieties or genotypes may be more resistant to the toxin than others. Butler's laboratory at Purdue is using *Striga* toxin as a screen for identifying clones of *in vitro* cultured sorghum cells with enhanced resistance to the toxin. They expose the cultured cells to various toxin concentrations and various periods of time. A few resistant cells survive while other are eliminated. The former are rescued on toxin-free medium and utilized to establish new clones. Already about 100 progenies of such selected and regenerated sorghum clones are being tested for enhanced *Striga* resistance in the field (Ejeta *et al.*, 1992).

Use of herbicides: The observation of prevention of differentiation of *Striga* embryos by 2,4-D application (and perhaps other herbicides) is significant for *Striga* control. Applied at the time of *Striga* germination would lead to abnormal seedlings unable to attach to host roots, which die. Further studies along these lines are called for, as well as employing aseptic culture methods as an initial screen for herbicides before selecting those with potential for field evaluation.

Nitrogen Fertilizers: Urea appears a good candidate for more critical studies on cultured parasitic weeds as well as application to cultured explants of host (sorghum or maize), and for tests in the field.

Biotechnology Approaches to Biology and Control of Parasitic Weeds.

Currently available biotechnological tools of isozyme, RFLP, & RAPD analyses offer new avenues for development of parasitic weeds control. Hosts (e.g. sorghum, maize, etc) genome analysis by molecular markers would yield new insights on linkage of traits of importance in their resistance to *Striga*. Fortunately, there are in progress projects in mapping of genomes in maize, sorghum and other host crops of *Striga*. Besides, success in aseptic cultures of tissues and cells of *Striga* and host plants will also enhance progress in transformation experiments through gene isolation, cloning and transfer. Also, in this domain is development of genetically modified (transgenic) crops for herbicide resistance (Joel et al. 1995). Furthermore, identification and characterizations of parasites developmental signals, coupled with synthesis of suitable analogues will lead to an integrated approach to control combining resistant host cultivars with chemical measures (such as seed germination stimulants, herbicides, nitrogen compounds) of control.

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Table 1

REVIEW OF ASEPTIC CULTURE STUDIES ON PARASITIC WEEDS

Plant Species	Family	Culture Medium	Response	References
Aeginetia indica	Orobanchaceae	MS + Sugar + Water melon Juice	Normal Plants. Flower: 4 -5 months	Tashima *et al.* (1974) French & Sherman 1976
Cistanche tubulosa	Orobanchaceae	MS + Sugar + CM-CH	Callus on germination Shoot bud on transfer to THS. No Plantlets	Rangan & Rangaswamy (1968)
Orobanche aegyptiaca	Orobanchaceae	MS + IAA or Kin or GA	Germination with radicle forming callus, or normal seedlings. No flowering	Kumar & Rangswamy (1977)
Striga hermonthica	Scrophulariaceae	MS + Sugar	Normal plants Flowering 4 - 5 months	Okonkwo (1964, 1966)
S. asiatica (red-flowered American Variant)	Scrophulariaceae	MS + Sugar + Kin + glut.	Germination normal plants, no flowering	Yoshikawa *et al* (1978)
S. asiatica (red-flowered American Variant)	Scrophulariaceae	MS + Sugar	Germination, normal plants. Flowering 3 months	Riopel & Baird (1987)
S. asiatica (white-flowered Indian Variant)	Scrophulariaceae	MS + Sugar	Germination, normal plants, Flower -3 months	Chidley & Drennan (1987)
S. asiatica (red-flowered American Variant)	Scrophulariaceae	MS + Sugar	Germination, normal plants, Flower -2 months.	Okonkwo (1991)
S. generioides	Scrophulariaceae	MS + Vitamins + Sugar	Normal plants Flower -2fi months	Okonkwo (1982)
Alectra vogelii	Scrophulariaceae	MS + Sugar	Normal plants, no flowering	Okonkwo (1975)
A. sessiflora var. *senegalensis*	Scrophulariaceae	MS + Sugar	Normal plants, no flowering	Okonkwo (1992)

III.14

ECOPHYSIOLOGY OF *Amyema mackayense*, A MISTLETOE ON A SALT EXCRETING MANGROVE HOST

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ABSTRACT

Amyema mackayense is a fairly common parasite of *Avicennia marina* in coastal mangrove communities of Southern Queensland, Australia. The mangrove host takes up salt from the saline soil solution, transports it to the leaves and excretes excess salt through special glands. The mistletoe has no mechanism to effectively prevent salt uptake from xylem water. Its way of coping with excess salt is a high turnover rate of leaves, which become succulent and even bloated before they are shed. Foliar salt content is extremely high in old leaves, when based on dry matter, but remains more or less constant throughout the lifetime of a leaf, when based on foliar water content. Yellowing of the leaves before shedding indicates re-translocation of nutrients into the branches, to conserve this resource.

Additional key words: sodium, chloride, salt accumulation, salt stress, succulence, adaptive strategy.

Drying of leaf samples: Since there was no drying oven available, samples had to be dried in the microwave oven of the camper van. It took some experimentation, because leaves tended to explode, seep slime, or char. The following procedure gave reasonable results. Leaves were intensely pricked with a needle, put in polyethylene bags, which were kept open on one side by wooden pegs, and dried for several hours at the lowest setting (defrost). Enough leaf-mass had to be in the oven to absorb microwave radiation and prevent overheating of the samples.

Laboratory analysis: Nitrogen and mineral elements were determined in the lab in Vienna on composite samples (4 replications each) according to standard laboratory procedures. Analytical error between replicates was less than 5 percent.

Statistical analysis: Statistical analysis could only be performed for leaf area as well as fresh and dry mass of leaves from pair 2 onward. Relative standard deviation ranged between 20 and 40 percent of the mean, which amounts to confidence intervals of less than +/- 10 percent.

Results

Fig. 2 shows the development of leaf area and leaf mass from the emergence of the leaves to their shedding. Leaf area increment peaks at leaf pair two, leaf mass increment at leaf pair four, indicating a pronounced increase in succulence with age. The leaf blade increases from a thickness of less than 1 mm to between 5 and 6 mm, before the leaf is shed.

Fig. 3 shows the mineral element content of leaf dry matter at various development stages.

Fig. 4 shows the increase in leaf water content during leaf development and the mineral content per unit leaf area, as well as the sodium chloride concentration in the leaf water. 28 g NaCl.l-1 amounts to about 480 mmol NaCl.l-1.

Discussion

This modest study strongly supports the hypothesis that dilution of salt by water uptake into ever swelling leaves is the key mechanism of *A. mackayense* to avoid excessive salt concentrations in its tissues on its salt excreting mangrove host. While leaf dry matter consists of about one third of inorganic solutes before the leaf is shed, sodium chloride concentrations in leaf water remain more or less constant at between 450 to 500 mmol NaCl per litre over the whole life span of the leaves, a value comparable to salt levels found in mangroves (Popp, 1984) and comparable to levels observed in halophytes (Flowers et al., 1986). It was not possible to measure turnover rates of leaves during the brief stopover at Poona, but the occurrence of freshly shed leaves on the sand below the Avicennia trees between each high tide indicates a fairly rapid turnover. The fact that leaves turn yellow at position 4 and are bloated and orange-yellow or pale at position 5 or 6 before they are shed, points in the same direction.

Popp et al. (1995), working on South African *Tapinanthus* and *Viscum* species on non-mangrove hosts, have postulated that succulence in mistletoes serves as a means to keep ion concentrations at a physiologically tolerable level, when high amounts of salt are supplied by their hosts. The results of this study of a mistletoe on its salt excreting mangrove host strongly supports these findings.

If nutrient elements are considered (Fig. 3 and Fig. 4), it becomes clear that thickening of the leaves is achieved with minimal expenditure of nitrogen and phosphorus, which seem to be limiting elements under the conditions encountered at Poona. Nitrogen is re-translocated back into branches and younger leaves before the defunct leaves are shed. Calcium content is quite low and increases with leaf age, as expected, but never reaches the high levels found in long-lasting leaves of mistletoes on host trees, which grow on non-saline soils (Glatzel, 1983). Potassium is accumulated towards the end of the life span of the leaves of the mistletoe. This

again is not surprising, considering the fact that mistletoes have a strong tendency to accumulate potassium (Glatzel and Balasubramaniam, 1987). The relatively modest accumulation of potassium points to rapid turnover rates of leaves once again.

In conclusion, this study has shown that even within the family Loranthaceae, considerable variation exists with regard to the adaptation to the physiology of the host trees. On trees growing on leached soils poor in salts, high transpiration rates and low photosynthetic carbon gain (Schulze et al., 1984, Glatzel and Balasubramaniam, 1987) seems to be the strategy of mistletoes to avoid outgrowing the nutrient supply provided by their hosts. Under conditions of high solute supply, modest transpiration, succulence, and, in this case, probably high turnover rates of leaves are the means to cope with potential damaging salt accumulation. Many interesting questions could not be addressed in this small study. Of particular interest would be an investigation of turnover rates of leaves in host and mistletoe, as well as carbon economy. Of equal interest would be a study on the organic solutes (Popp, 1983) in this mistletoe host association.

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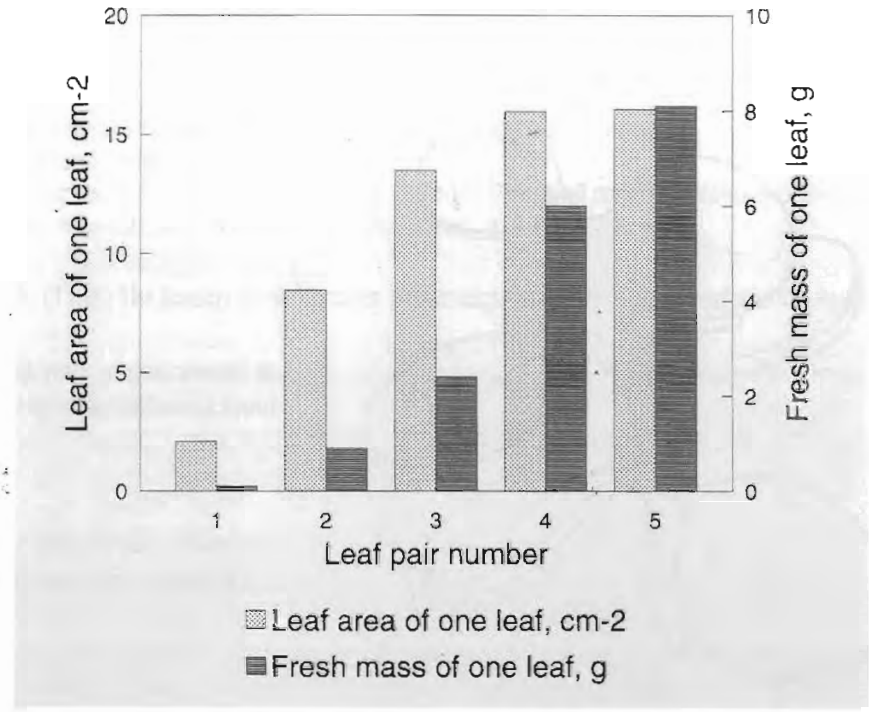


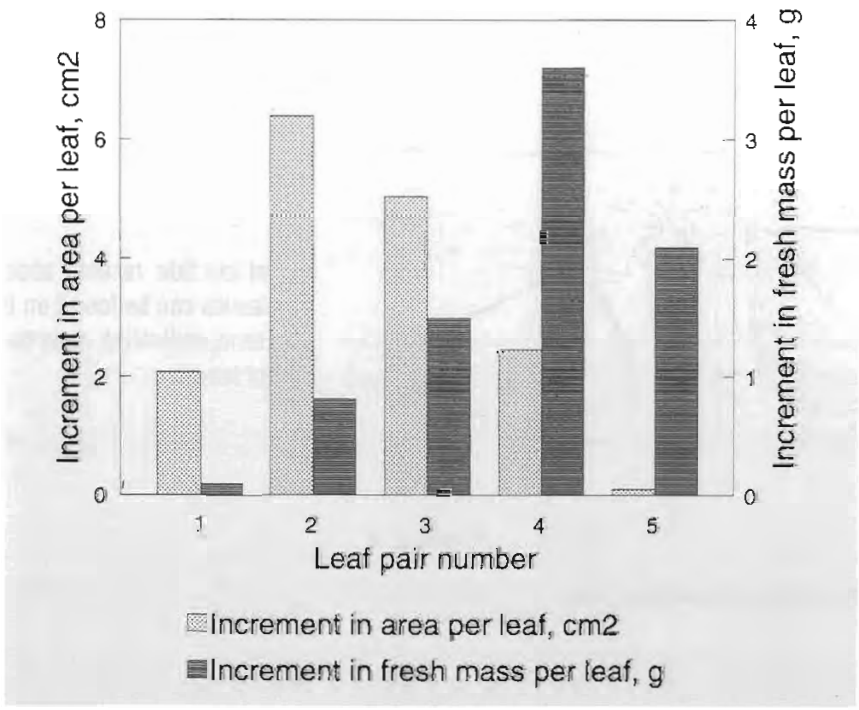
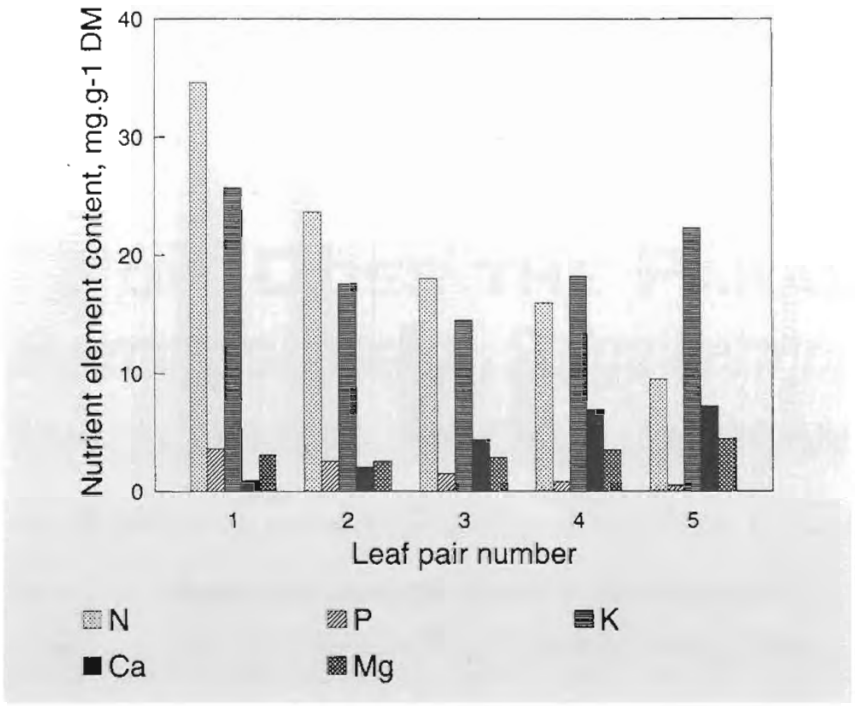


Figure 2

Development and increments of leaf area and leaf fresh mass in *Amyema mackayense* from leaf pair number one to five



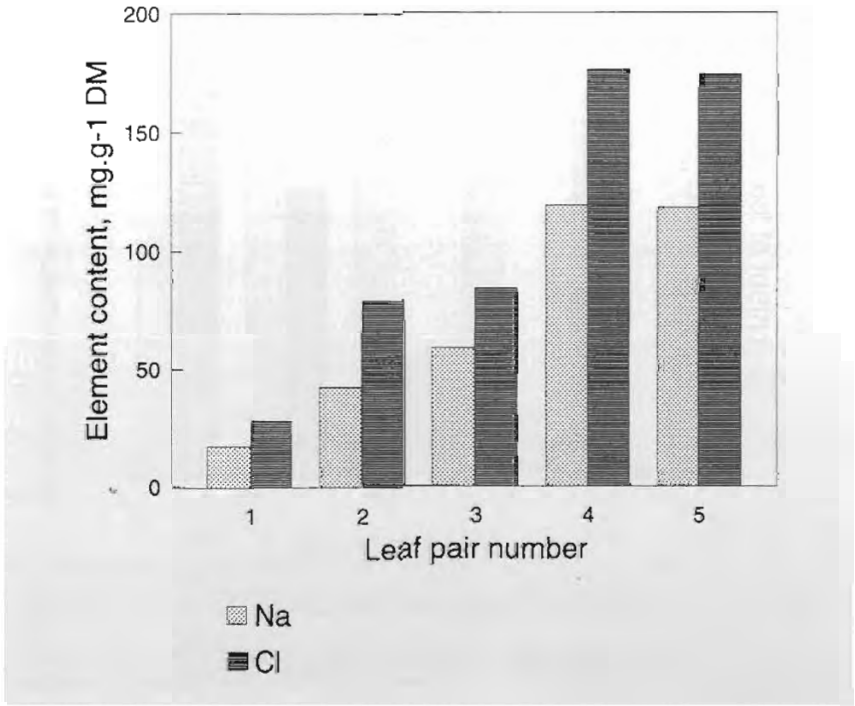


Figure 3

Mineral element content of leaf dry matter of *Amyema mackayense* at various leaf development stages

Introduction

Parasitic weeds are dependent on their host plants, to varying degrees, for the supply of water, inorganic and organic solutes. The removal of these resources by the parasite can limit the growth of infected plants, such that dry matter accumulation is lower than for uninfected plants grown under the same conditions. Differences in biomass accumulation and yield between infected and uninfected plants may result either directly from the loss of resources to the parasite, which represents a new sink, or indirectly through other processes resulting from the presence of the parasite and the concomitant removal of resources. Hibberd *et al.* (1996a,b) show that for associations of *Orobanche aegyptiaca* and *Striga gesnerioides* with broad leaf hosts, differences in biomass accumulation between infected and uninfected plants may be explained in terms of the parasite imposing an additional sink on the host. In this paper we suggest that *S. hermonthica* influences biomass accumulation and allocation in both C_3 and C_4 cereals in ways which can not be explained with respect to sink demand, and demonstrate that the extent to which the parasite influences the host is dependent on both host genotype and environment, for example, nitrogen supply.

Effects on Host Growth

Striga hermonthica (Del.) Benth. (from a sorghum host) exerted a marked influence on host growth and allometry almost immediately following infection. The distance from the base of the stem to the youngest fully emerged ligule on 28 day old plants (two sorghum genotypes (CSH1 and Ochuti) and one rice genotype) was significantly lower on infected cereals compared to uninfected controls within 4 days from the first observation of *S. hermonthica* plants on the host root system (Fig. 1). At this stage the young *Striga* plants were approximately 1-2 mm in length. Careful monitoring of their development was possible since plants were grown in sand in a rhizotron system. (This comprised two perspex sheets, 30 x 40 cm, separated by a 1 cm gap, filled with sand and with a drip-feed nutrient supply). There was a good correlation between the influence of *S. hermonthica* on cereal stem length and stem dry weight. At this stage the biomass of the parasites was negligible compared to the difference between uninfected and infected plants. The difference between heights of infected and uninfected plants increased with time, and by 48 days for sorghum and 41 days for rice, the height of infected plants was between 22% and 44% lower than that of control plants. Total biomass of both infected sorghum cultivars was approximately 30% lower than that of the controls, whilst infected rice plants were half the biomass of uninfected plants. A marked shift in allocation of dry matter to roots was also observed (data not presented).

When the plants were harvested, *Striga* had not yet emerged above the soil surface, and the biomass of parasite tissue was negligible in comparison to that of the host. Both sorghum cultivars supported the same mass of *Striga* tissue (0.24 g dry weight), whilst rice supported considerably less (0.05 g dry weight). Given that the effects of the parasite are so dramatic in the days following infection, and that the final biomass of *Striga* was so low in comparison with the difference in dry weight between infected and uninfected cereals, it is unlikely that we can account for the effects on the host in terms of the parasite only imposing an additional sink for carbon and nutrients. In particular the effects on rice were so severe that the parasite caused the death of the host by 45 days after planting. These findings contrast with those for associations of dicotyledonous plants with *S. gesnerioides* and *Orobanche aegyptiaca* (Hibberd *et al.* 1996a,b; Barker *et al.* 1996), where differences in biomass accumulation between infected and uninfected plants are of a magnitude which can be accounted for by sink demand by the parasite.

Effects on Host Photosynthesis

In addition to growth and allometry, gas exchange measurements were made on the leaves of infected

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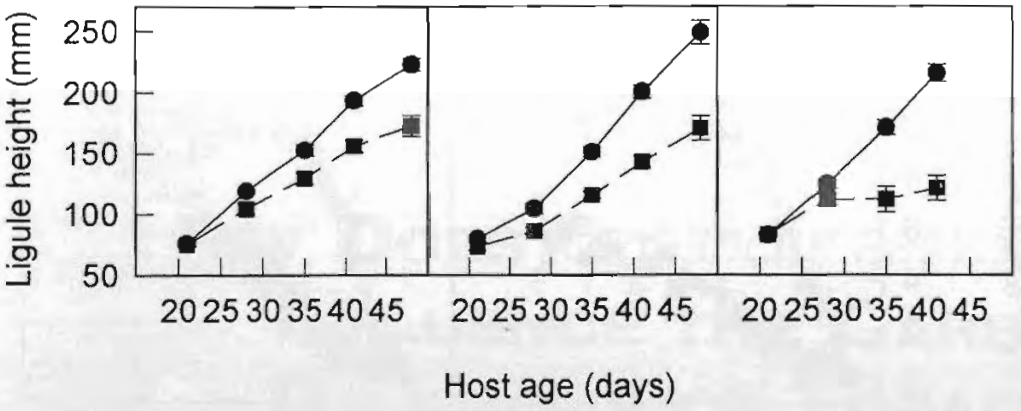

Figure 1

Height of stem from base to youngest fully emerged ligule for two cultivars of sorghum (CSH_1 and Ochuti) and rice, in the presence (■) or absence (●) of. *S. hermonthica* (± s.e).

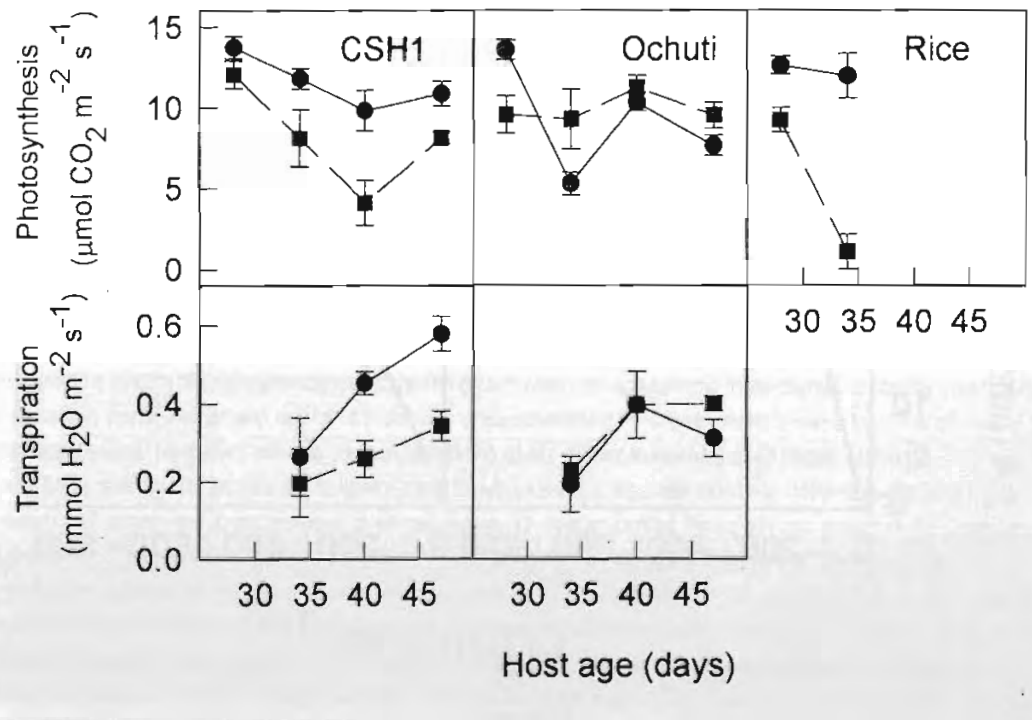

Figure 2

Rates of photosynthesis and transpiration in two cultivars of sorghum (CSH1 and Ochutti) and rice, in the presence (■) or absence (●) of. *S. hermonthica* (± s.e).

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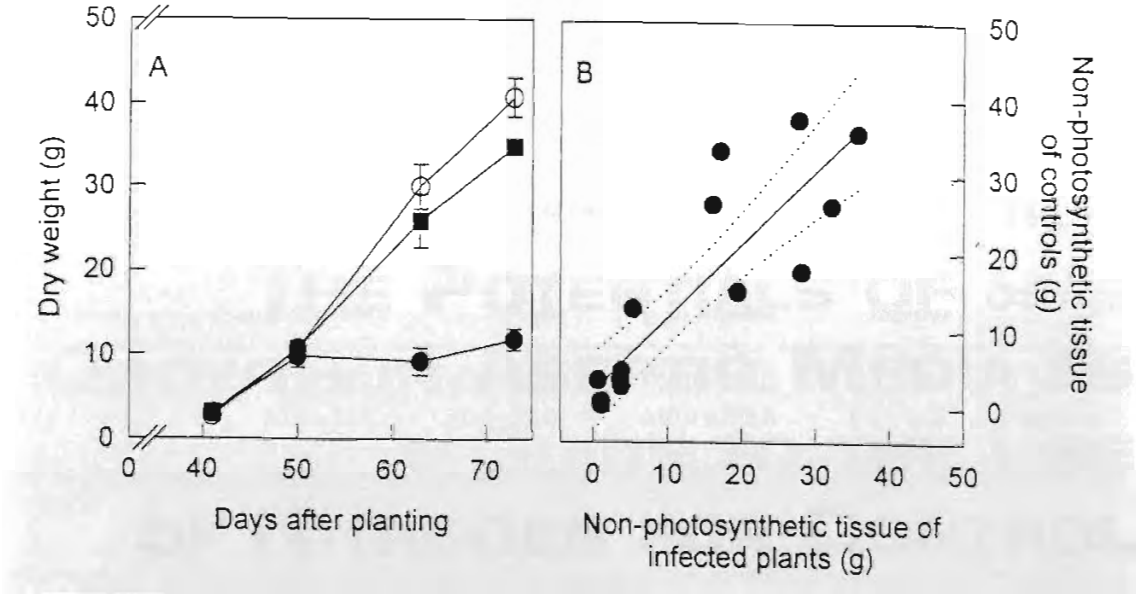

Figure 1

1A. The dry weight of tobacco plants from 40 to 73 d after planting. Tobacco were either unparasitised (O) or parasitised by *Orobanche aegyptiaca* (●), also indicated is the combined dry weigth of the host and the parasite within the infected system (■). 1B. The amount of non-photosynthetic dry matter in control plants (flowers, stem, and roots), plotted against the amount of non-photosynthetic dry matter in the infected system (flowers, stem, roots and *Orobanche*).

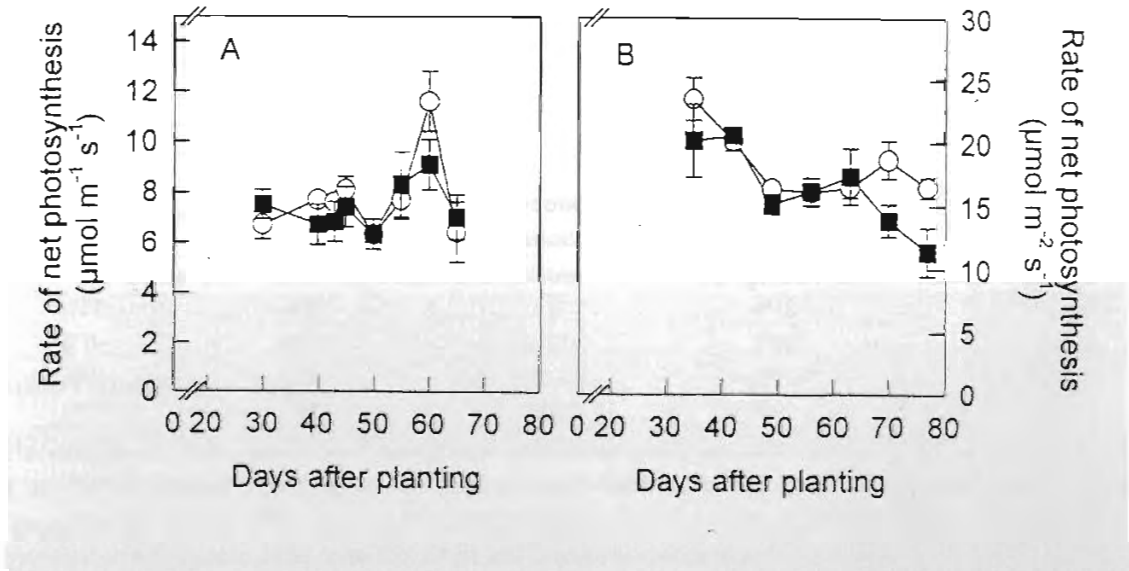

Figure 2

The rate of net photosynthesis (µmol m^{-2} s^{-1}) in the youngest fully expanded leaves of tobacco and tomato plants. **A:** Uninfected tobacco plants (O) and tobacco plants parasitised by *Orobanche* (■) (measured at 500 (µmol PFD m^{-2} s^{-1}). **B:** Uninfected tobacco plants (O) and tobacco plants parasitised by *Orobanche* (■) (measured at 1200 (µmol PFD m^{-2} s^{-1}). Data are shown as means ± s.e.

Introduction

A reliable interpretation of *Striga* response to several control strategies, e.g. use of nitrogen fertilizers, has been hampered in the past partly because of the confusing role, interaction, and influence of the host plant. Ability to grow *Striga* plants alone until flowering in aseptic media removes this constraint.

It has been reported in the past that nitrogen fertilizers suppress the severity of *Striga* attack while simultaneously increasing host yield (Okonkwo, 1991). Using the aseptic culture technique, it can be shown that nitrogen fertilizers exert their influence on *Striga* by directly affecting the parasite seed germination, growth and development (Igbinnosa *et al.*, 1996). Low presence of inducible and non-inducible nitrogen assimilation enzymes, leading to an accumulation of ammonium and nitrate ions to toxic levels, is reported as being part of the underlying physiological factor responsible for nitrogen action (Igbinnosa and Thalouarn, 1996).

This paper aims at looking at the potentials of *Striga* growth in aseptic media in host abence. It also looks at the nitrogen effect on nitrogen assimilation enzymes in *Striga* , and for the first time reports the presence of the NR gene in *Striga* spp.

Materials and Methods

Source of plant materials and growth conditions. *S. hermonthica* (Del.) Benth. seeds were collected from a maize farm in 1990 in Mokwa, Zaria, Nigeria. Methods of seed sterilization, preconditioning in water, seed germination in synthetic stimulant, media preparation, growth of *S. hermonthica* in aseptic media, and growth of parasite and host in pots were adapted from Igbinnosa (1993). *S. hermonthica* seedlings were grown in aseptic media in a growth chamber with 12:12 hrs light and dark cycles with light intensity of 100 mol.$^{-2}$ s^{-1}. Screenhouse condition was 16 hrs per day with light intensity of 200 mol. m^{-2} s^{-1} of photosynthetic active radiations.

DNA extraction and purification and probe preparation. The cloned nitrate reductase (NR) gene probe was prepared from tobacco and generously provided by Dr C. Meyer (INRA, Versailles, France). DNA purification and probe labelling were performed as previously described (Thalouarn *et al.*, 1994).

DNA amplification and Southern hybridization were conducted as described in Thalouarn *et al* (1994).

Vaucheret *et al.* (1989) cloned and characterized tobacco NR structural genes, and identified 4 exons of 1012, 141, 233 and 1326 base pairs (bp) each. Due to difficulties encountered in amplifying the large NR gene from tobacco (6087bp) as a whole, amplification of individual exons was decided as a solution to this problem. This report is therefore based on the amplification of the exon of 1012 base pairs (figures 1 and 2).

Culture of S. hermonthica seedlings. Using $(NH_4)_2SO_4$,and KNO_3 as NH_4^+ and NO_3^- sources respectively, *S. hermonthica* seedlings were grown in media containing 60mM NH_4^+ and NO_3^- as sole nitrogen sources at ratios 0:1, 1:3, 1:1, 3:1 and 1:0. Seedlings were grown for 15 days in continuous darkness and transfered to another growth chamber with 12 :12 hrs light and dark cycles for another 25 days.

Determination of NR activity (NRc) in plant tissues. Method of NRc measurement on plant leaf strips was as previously described by Hunter and Visser (1986). Each treatment had 3 replicates and NRc was expressed in enzymatic units, with one unit (U) equal to the quantity of enzyme which catalyses the transformation of 1 mole of substrate per hour and per gram fresh weight. For control, KNO_3 was replaced with distilled water.

Statistical analysis. All experiments were repeated 2 to 3 times, with each having 3 replicates. Data collected were analysed using the Least Significant

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Table 1

A COMPARISON OF NITROGEN ASSIMILATING ENZYME ACTIVITIES IN *STRIGA HERMONTHICA* GROWN IN ASPETIC MEDIA AND IN POTS

Growth media	NRc (U)	GSc (U)	GDHc (U)
Aseptic media	1.41	1.48	5.63
Potted soil	0.38	2.06	1.34
+N	0.63	-	-
maize host	6.89	36.12	2.92
LSD			
5%	0.01	1.53	0.63
1%	0.02	2.54	1.05

+N = 30 mM NH_4NO_3 and 30 mM KNO_3 solution added to soil in pot.
Dashes indicate that no data were collected.

Table 2

EFFECT OF NH_4^+ AND NO_3^- RATIOS ON THE DEVELOPMENT AND NITRATE REDUCTASE ACTIVITY IN *STRIGA HERMONTHICA* PLANTS GROWN IN ASPETIC MEDIA

NH_4^+ and NO_3^- ratio	Total fresh weight (mg)	NRc (U)
0 : 1	20.98	0.68
1 : 3	61.00	1.24
1 : 1	46.25	1.53
3 : 1	33.70	0.89
1 : 0	0.00	0.00
control (1 : 2)	55.60	1.49
LSD		
5%	14.85	0.06
1%	20.34	0.08

Introduction

Striga spp. are a significant constraint to crop production particularly in sub-Saharan Africa. *Striga* is especially problematic for the small-scale farmers who have limited resources, limited access to inputs and limited flexibility in the crops grown. Furthermore, hand-weeding, the traditional method of controlling weeds, used by the small-scale farmer, is ineffective since a significant amount of crop damage caused by *Striga* occurs before it emerges from the ground. The crop losses caused by *Striga* can be considerable, and far exceed losses that can be explained by competition for resources by the parasite (Parker and Riches, 1993). This suggests that, in addition to competing for water, nutrients and carbohydrates, *Striga* exerts a potent toxic effect on the host. Symptoms of *Striga* parasitism such as stunting, chlorotic and necrotic lesions and wilting under well-watered conditions support the concept that *Striga* is phytotoxic to its host. Furthermore, crude extracts of *Striga* leaves were found to induce chlorophyll loss and wilting when injected into the stem of susceptible hosts (Ejeta and Butler, 1993).

There are few quantitative data available on the magnitude of *Striga*'s phytotoxic effect on maize, particularly from field-based experimentation. Understanding the relative importance of the competitive and phytotoxic effects of *Striga* can provide direction on the type of research that should be undertaken to understand and control *Striga*-induced crop losses.

The objective of this study was to estimate the phytotoxic or non-competitive effect of *Striga* on maize from field-based experiments.

Materials and Methods

Data for this study were obtained from field experiments conducted in the USA (temperate environment), Kenya (mid-altitude tropical environment) and Ivory Coast (lowland tropical environment) during the period 1989 and 1992. Experiments that were selected for inclusion in this study met the following criteria: precise measurements of *Striga* dry weight and crop yield were taken, and the range in the level of *Striga* infestation within the experiment was large. In the experiments included from the USA, involving *S. asiatica* (L.) Kuntze, treatments focused on methods of *Striga* seed augmentation; two of the sites had previously been fumigated with methyl-bromide to allow for greater control in the levels of infestation. In the Kenya experiment, treatments were: natural levels of *Striga hermonthica* (Del.) Benth. seed , artificially augmented levels of *Striga* seed, and application of ethylene gas 3 weeks after planting to reduce the natural level of *Striga* seed in the soil. Finally, the treatments in the Ivory Coast experiment consisted of eight genotypes which varied in their response to *S. hermonthica* with and without artificial augmentation. Additional details on each of these experiments are summarized in Table 1.

In all experiments, *Striga* dry weight was determined by hand-pulling and oven-drying all emerged *Striga* plants shortly after the most mature plants began to senesce. At this time, maize was in the late grain-filling stage (approximately 100 days after planting). There was no significant emergence of additional *Striga* after this initial harvest, so no additional harvests were carried out. Maize grain yield was obtained by harvesting the bordered center of the plot in all locations except Ivory Coast, where the complete plot was harvested. Moisture content was determined from a 500 g sample of shelled grain. Total above-ground maize biomass was estimated from the dried grain weight using a harvest index of 0.40, as no actual measurements of maize stover were taken. The same net plot area was used for determining both *Striga* dry weight and maize grain yield. For ease of interpretation and presentation all data were converted to g/m^2.

In order to estimate the effect of *Striga* dry weight on above-ground maize biomass, linear regression equations were calculated for each experiment with *Striga* dry weight/m^2 as the independent variable

and above ground maize biomass/m^2 as the dependent variable. Correlation coefficients for these two variables were also calculated.

Results

There was a good range in *Striga* development within all experiments included in this study (Table 1). Although, *Striga asiatica* is generally smaller than S. *hermonthica*, the range in *Striga* dry matter production was similar for experiments infested with S. *asiatica* and S. *hermonthica*. There were large differences between maize productivity both within and between experiments (from 0 g/m^2 at Homa Bay to 1887 g/m^2 at Dillon-early planted) indicating that the selected experiments reasonably represent a wide-range of maize production environments.

Striga dry weight/m^2 and above-ground maize biomass/m^2 were significantly and negatively correlated in all experiments (Table 2). The slopes of all regression equations were significantly different from zero ($p \leq 0.05$, data not shown). In these equations, the y intercept *(a)* represents the estimated above ground maize biomass/m^2 under *Striga*-free conditions and the slope *(b)* represents the rate of above ground maize biomass reduction (in g/m^2) for every g/m^2 of *Striga* dry matter produced. In a completely competitive relationship, the slope of these equations would be equal to negative 1.0. In these experiments, the rate of the estimated above ground maize biomass reduction per g/m^2 *Striga* growth varied between -7.7 g/m^2 to -14.4 g/m^2. This means that between 7% and 13% of the total biomass reduction in maize caused by *Striga* can be attributed to competitive effects (since the slope of a competitive relationship is equal to 1.0, the inverse of the estimated slope from field data will equal the percent reduction attributable to competition). The greatest rate of reduction occurred in the highest yielding experiment at Dillon. The ranges in the rate of above-ground maize biomass between experiments with *S. asiatica* and those with *S. hermonthica* were similar.

Discussion

Using regression analysis based on field experimentation, we found that *Striga* caused 7.7 to 14.4 times more above-ground maize biomass loss than can be explained by competition for resources. Cechin and Press (1993), from pot experiments, reported sorghum biomass reductions, including root mass, similar to those we found (-15.22 and -14.48). Parker (1984), during the initial stages of parasitism reported an even greater phytotoxic effect (at least 30 times the weight of the parasite).

We did not attempt to include the below ground biomass of *Striga* in the calculation of regression equations. Therefore, the effect of competition is probably underestimated. However, the dry weight of a mature *Striga* plant can be 40 times the weight of a typical subterranean plant (G. van Delft, personal communication). Based on these observations, we have assumed that the addition of non-emerged *Striga* would not significantly alter the results of this study (less than 10-20%). Nevertheless, as better field data on the relative proportion of above and below ground *Striga* dry weight near the end of the season are available, these regression equations should be adjusted accordingly.

Although no toxin from *Striga* has yet been identified, the effect of *Striga* on a number of physiological parameters of the host has been quantified. Host plants that are parasitized by *Striga* generally partition more of their dry weight into their roots. Patterson (1990), in glasshouse and growth chamber studies, found significant *Striga* induced increases in the root/shoot ratio in maize. Based on his data, the increased partitioning of dry weight to the root caused by *Striga* parasitism, would result in the reduction in shoot biomass of 21-31%. Since the economic portion of the plant in cereals is the above-ground portion (primarily the grain and to a lesser extent the stover), any *Striga*-induced changes in partitioning away from the shoot must be considered undesirable.

Photosynthesis in the host is also significantly reduced by *Striga*. From a field experiment in western Kenya, Gurney *et al.* (1995) found a 31% reduction in photosynthesis in maize infested with *Striga* compared with the control at 63 days after planting. This *Striga* induced decline in photosynthesis progressively increases with time after parasitism. The combined effects of increased partitioning to the roots and the decreased rate of photosynthesis can explain about 60% of the above ground losses caused by *Striga*. Since the measured phytotoxic effects of *Striga* averaged 90% of the total *Striga* related losses, additional physiological processes other than partitioning and photosynthesis are also negatively affected.

These data verify results of others working in more controlled environments, that *Striga* does exert a potent phytotoxic effect on its host. Because of the magnitude of the yield losses caused by these phytotoxic effects relative to *Striga*'s competitive effect, addition research on identifying the toxin(s) produced by *Striga* and their mode of action is justified. The data also suggest that breeding for tolerance to *Striga*, although less desirable than breeding for resistance as resistant cultivars will produce less seed, would result in yield improvements nearly as great (within about 10%) as those that could be achieved through breeding for resistance. Tolerant varieties may also assist farmers in reducing *Striga* seed banks by enabling them to increase farm output in the short term, and thereby providing them with more resources and incentive to invest in *Striga* control, such as hand-weeding. Good tolerance to *Striga* in sorghum has been observed. Gurney *et al.* (1995) found no significant yield loss under high levels of *Striga* in a tolerant local variety "Ochuti". Interestingly, photosynthesis was not effected by *Striga* parasitism in this variety. In sorghum, Ejeta and Butler (1993) have also found variability for tolerance to extracts of *Striga* and are currently using tissue culture media containing *Striga* extracts to select for tolerant types. If the principal toxins could be identified and isolated, this work could proceed more efficiently.

Unlike strictly competitive effects, phytotoxic effects are cumulative. We have observed that early *Striga* parasitism is much more damaging than later parasitism. The more than three fold increase in yield of sorghum compared to maize in an experiment in western Kenya was thought to be related to the 2 week delay in *Striga* parasitism of the sorghum as the final levels of parasitism were similar (Ransom and Odhiambo, 1993). Berner *et al.* (1995) also found large increases in productivity with delayed parasitism in both maize and sorghum. Management practices that delay attachment of *Striga* will aid in minimizing yield losses and should be developed. Low doses of herbicides dressed to seeds of maize cultivars, including those that are herbicide resistant, may be one such management practice that can be used to reduce the effects of early parasitism (Ransom *et al.*, 1995 and Berner *et al.*, 1995).

Acknowledgment

The financial support of the Canadian International Development Agency (CIDA) through the Eastern Africa Cereals Project is gratefully acknowledged.

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Table 1

DETAILS OF EXPERIMENTS USED TO ESTIMATE THE RELATIONSHIP BETWEEN ABOVE-GROUND MAIZE BIOMASS AND *STRIGA* BIOMASS

Location/Date	Species	Trial type	Range *Striga biomass* (g/m^2)	Range Maize *biomass* (g/m^2)
Bouake, Ivory Coast, 1992, n=32[1]	S. hermonthica	8 inbred lines with and without artificial Striga.	0-34	65-886
Homa Bay, Kenya, 1990, n=68	S. hermonthica	Natural, augmented, ethylene at 3 wks.	5-53	0-910
Evergreen, NC., USA, 1989, n=42	S. asiatica	Natural and augmented.	0.1-33	23-500
Dillon, S.C. USA, 1989, n=21	S. asiatica	Fumigated, artificially infested, early planted.	1-36	789-1887
Dillon, S.C., USA, 1989, n=17	S. asiatica	Fumigated, artificially infested, late planted	4-45	121-642

1. n = the number of observations in each experiment used in the regression analysis.

Table 2

LINEAR REGRESSION EQUATIONS ESTIMATING ABOVE GROUND MAIZE BIOMASS (Y) FROM STRIGA DRY WEIGHT (X), THE STANDARD ERROR OF THE SLOPE (B) OF THESE EQUATIONS AND CORRELATION COEFFICIENTS BETWEEN ABOVE GROUND MAIZE BIOMASS AND STRIGA DRY WEIGHT, FROM DATA FROM FIVE EXPERIMENTS

Experiment	*Regression equation*[1]	*Standard error b*	Correlation *coefficient*[2]
Ivory Coast	y=539-12.5x	3.26	-0.57**
Homa Bay	y=709-12.0x	2.57	-0.50**
Evergreen	y=302-8.3x	2.19	-0.51**
Dillon early	y=1660-14.4x	5.81	-0.49*
Dillon late	y=604-7.7x	1.75	-0.75**

1. y = above ground maize biomass (g/m^2), x = *Striga* biomass (g/m^2).
2. **, * indicate statistical significance at the 1% and 5% level, respectively.

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Table 6

MAIZE PRODUCTION IN MALAWI AFFECTED BY *STRIGA ASIATICA* AND TOTAL YIELD LOSSES DUE TO THE PARASITE

Districts	Mean infection intensity of all fields surveyed (%)	Yield losses induced by *Striga* (%)	Potential yield without *Striga* (tons)	Total yield losses (tons)
Ngabu	12.9	3.6	46,684	1,681
Blantyre	18.5	5.2	196,061	10,195
Liwonde	20.4	5.7	269,790	15,378
Salima	3.8	1.1	89,207	981
Lilongwe	27.8	7.8	546,731	42,645
Kasungu	27.4	7.7	377,661	29,080
Mzuzu	13.5	3.8	147,192	5,593
Karonga	2.9	0.8	21,778	174
Total	**16.0**	**4.5**	**1,695,104**	**105,727**

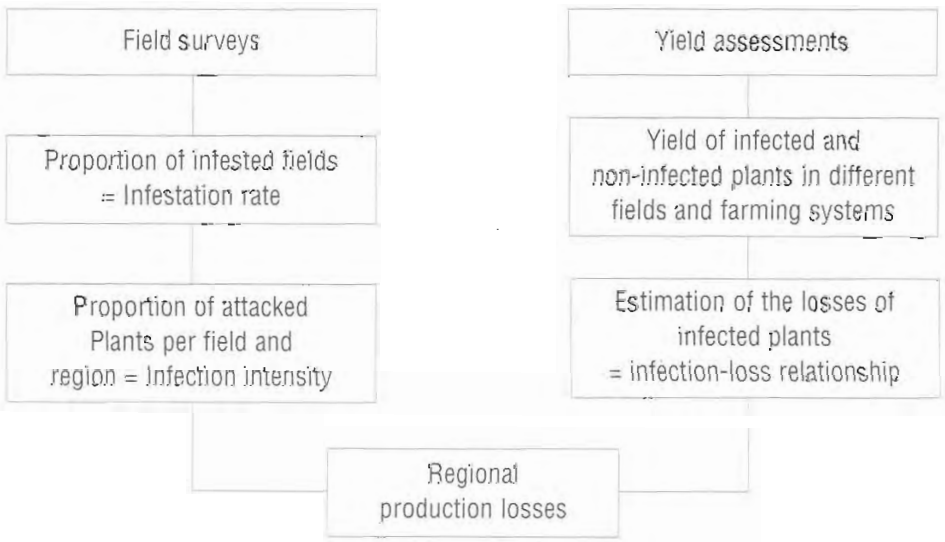

Figure 1

Model for the estimation of regional losses caused by parasitic weeds

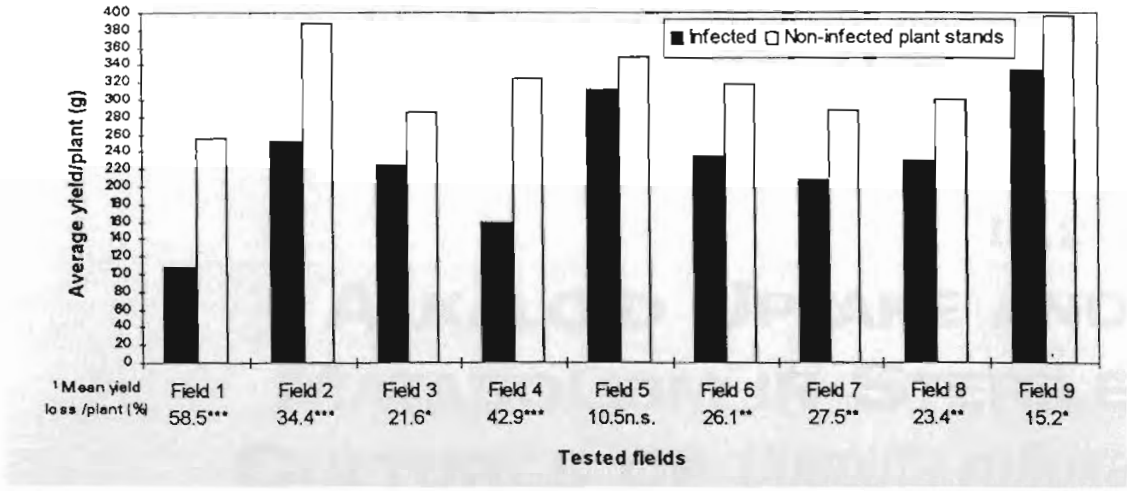

Figure 2

Influence of *Striga*-infection on maize yield in Malawi 1994, determined in single fields comparing the yield of infected and non-infected plant stands ([1] according to Walker's formula, 1983).
(Significance: [*] $0.05 \geq p \geq 0.01$; [**] $0.01 \geq p \geq 0.001$; [***] $p \leq 0.001$).

nitrogen specific detector (NPD at 310C). All alkaloids were detected using a temperature programme, starting with 100C (2 min isothermal, 15C/min to 250C, 20C/min to 300C, 10 min isothermal).

Results

Shoot and callus of *Cuscuta reflexa* are able to grow on nitrogen-free liquid growth media for maximal eight weeks without alkaloids. After this time green callus becomes pale and brown and soon died, but shoot is furthermore growing only at the apex while dying at the distal end. When adding alkaloids to the growth medium, callus and shoot keep almost normally green and show good growth and an increase of biomass for more than eight weeks. After alkaloid extraction all fed alkaloids can be detected in *Cuscuta*, in the shoot always a little more (21%) than in the callus (18%). One explanation for this observation may be that callus tissue is not differentiated and there is no possibility for transporting the alkaloids (Fig.1). When feeding alkaloids of a higher molecular weight, such as hyoscyamine, quinine, crotaline and strychnine, no metabolites of these substances can be detected. But when offering low-molecular alkaloids, such as nicotine and cytisine to *Cuscuta*, metabolites of the respective compounds can be found. Metabolites of nicotine are dehydronicotine (2%) and cotinine (5%), but conversely when feeding cotinine no nicotine can be detected. The same effect can be seen when feeding cytisine: the metabolites detected are N- methylcytisine (2%), dehydrocytisine I+II (together 2%) and N-formylcytisine (34%), but offering N-methylcytisine to the parasite, no metabolism is observed. These results confirm that physiological mechanisms (presumably enzymatical reactions) proceed in the parasite, since these metabolites can only be found in the tissue of the parasite and not in the liquid control medium without plant material, so *Cuscuta* shoot and callus apparently are able to metabolize some alkaloids.

Cuscuta takes up the alkaloids out of liquid media without any selection. This was confirmed by feeding an alkaloid-mixture with equal concentrations of nicotine, hyoscyamine and quinine. Just after one hour incubation all respective compounds can be detected in the parasite (Fig.2). After one day the alkaloid concentration in *Cuscuta* tissue has reached a level which remained constant during the next two weeks (Fig.2). Then cotinine can also be proved in traces beside nicotine (7%), hyoscyamine (22%) and quinine (71%). But when the plant material was put into alkaloid-free medium after feeding with this alkaloid-mixture for 14 days, a part of the accumulated alkaloid concentration was found in the medium again. Presumably a chemical balance between uptake and delivery of the respective compounds from tissue to medium is built up. Hyoscyamine and especially quinine have been accumulated in higher concentrations than nicotine and are still traceable after the sixth passage into alkaloid-free medium, while nicotine and its metabolites disappear just after the first change and cannot be proved after further passages (Fig.3). Since nicotine can always be found only in lower concentrations (10-15% of total concentration), this might be a sign that *Cuscuta* is able to use this alkaloid as a further nitrogen source.

In order to find out localization of the alkaloids inside *Cuscuta* cells berberine was offered to the parasite. After three days of incubation the yellow colour of the medium, resting from berberine was gone. Microscopical analysis (fluorescence at 366 nm) showed that berberine could be found in the apoplast (cell wall and xylem elements), but was also incorporated inside the protoplasm of the cells. There berberine seemed to be localized in the cytoplasm and not as expected inside the vacuole. We mostly can find one thick yellow particle per cell, often in the region of the nucleus, also noticeable in light microscopy , too (pictures not shown).

Discussion

As already described for host-parasite-interactions (Czygan *et al.*, 1988; Bäumel, 1994) *Cuscuta* is able to take up and accumulate alkaloids not only out of

its hosts via xylem and especially phloem, but also out of liquid growth media in sterile cultures (Bäumel, 1994; Ehrenfeld, 1994). The mechanisms of this uptake are still unknown, but there must also be an active transport into the cytoplasm. This speculation is supported by the observation that berberine, which at a pH of 5,8 in the medium exists as a protonized ion, can be accumulated in the cells, hence diffusion cannot be the only mechanism. Otherwise since there is no selection of the alkaloid uptake out of hosts and growth media, the mechanism cannot be specific (Bäumel, 1994; Ehrenfeld, 1994).

Wink and Witte (1985) could show that lupin seedlings are able to use the accumulated quinolizidine alkaloids as a nitrogen source for growth. *Cuscuta* shoot and callus without any further nitrogen source except alkaloids can also grow, especially when they are fed with low-molecular alkaloids such as nicotine and cytisine where we can find metabolites of the respective compounds. This metabolism might only be an act of detoxification but while observing growth we think it might be more an attempt to use the nitrogen component of the alkaloid-molecule for its own physiological metabolism. This speculation is also supported by the fact that especially nicotine can only be detected in the plant tissue in very low concentrations (10-15%) and sometimes was no more detectable in the tissue, compared with its concentration at the beginning of the experiment, but also cannot be found in the medium again (Bäumel, 1994; Ehrenfeld, 1994). So we speculate that *Cuscuta* shoot and callus are apparently able to metabolite and use nicotine as a nitrogen source.
The localization of berberine in *Cuscuta* cells was very astonishing for us because we expected the alkaloids to be accumulated in the vacuole. But Tabata (1991) showed that plants which themselves cannot produce alkaloids of a special type are mostly unable to transport the accumulated alkaloids into their vacuoles, the normal place of accumulation, and so must be placed in the cytoplasm. For *Cuscuta* it seemed to be the same situation. Berberine is also known as an antibiotic substance which is able to intercalate inside the DNA of bacteria (Tabata, 1991). Perhaps this observation might be an explanation for the phenomenon that we mostly can find only one berberine particle per cell, apparently located in the region of the nucleus.

Acknowledgments

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Introduction

The sieve tube connection from the haustorium of the holoparasite *Cuscuta reflexa* Roxb. to the host is established by a highly differentiated hypha cell on the tip of the haustorium; the so-called absorbing cell. It clasps, with fingerlike protrusions, around the host sieve tube. The common wall shows labyrinthic structures which are orientated towards the lumen of the hypha (Dörr, 1972). There are no sieve pores or plasmodesmata in this region, so that the transfer of substances from the host phloem to the parasite requires transport through the apoplast. This transfer comprises at least three processes:

1. The release of substances from the host phloem into the apoplast between the sieve tube and the absorbing hypha. This step appears to correspond to an apoplastic unloading, which has been described in the petioles of celery and in the stalks of sugarcane. However, the release of substances in these plants occurs in typical sink organs, which are programmed by the ontogenetic development. The attack by *Cuscuta* is, more or less, accidental. The site of the release is not predetermined.

2. The transfer through the apoplast. This could be connected with some metabolic conver-sion of the released substances, for instance, sucrose hydrolysis by extracellular invertase.

3. The uptake of the released substances and/or their metabolites into the absorbing hypha.

Until now the mechanism of the release of substances from the host sieve tube is, to a large extent, unclear. Wolswinkel and Amerlaan (1983) concluded from their results that the release of sucrose is accomplished by a specific carrier and under metabolic control. However, the release is not only restricted to the biotic substances. It was found to be the case for all compounds translocated in the host phloem including alkaloids and xenobiotics. In contrast to biotic substances, the membrane transport of these compounds is accomplished by diffusion. To compare the extent of the release of biotic and xenobiotic substances, we investigated the transfer of these compounds after their simultaneous application to the host.

In *Vicia faba* L. the attachment of *Cuscuta* is connected with an increase in the invertase activity in the host tissue that seems to be similar to the infection of mesophyll cells with brown rust (Strobel *et al.*, 1969, Tetlow and Farrar, 1993). We investigated whether an increase in the invertase activity is a prerequisite for the transfer of assimilates.

Materials and Methods

Plant material. *C. reflexa* parasitising *Pelargonium zonale* L. was cultivated in the green-house at 18-24C and 60-80 % relative humidity with a daily regime of 16 h light (daylight + fluorescence lamps) and 8 h darkness. For the experiments a well-grown *Cuscuta* shoot was cut off and affixed to the middle part of petioles of *P. zonale* and *Primula obconica* L., respectively. 16 days later the plants or their excised petioles were used for the experiments.

Translocation of $^{14}CO_2$-derived assimilates. 24 h before the translocation experiments with $^{14}CO_2$-photosynthates the host-parasite-systems were transferred to a growth chamber (15 h light, 17-165 $Em^{-2}s^{-1}$) at 20C and 9 h darkness at 12C. A part of the parasitised host leaf was exposed to $^{14}CO_2$ for 5 min. After 3 h the parasitised petioles were divided into the different parts. Radioactivity of methanol extracts was determined by liquid scintillation counting (LSC).

Qualitative and quantitative determination of sugars by HPLC. Methanol extracts of the different parts of the host-parasite-systems were lyophilised, dissolved in tridest water and purified by ion exchange (AG 50W-X4/ AG3W-X4 Bio-Rad USA). For detection on HPLC several columns (Biorad HPX 87-C, ERC-CA8S-100, Polysper CH-CA) were used.

experiments. The enzyme activity strongly depends on the age of the tissue. In young petioles of *Pelargonium* an infection of *Cuscuta* can induce an amplification of the normal enzyme gradient along the petiole. However, an increase in the enzyme activity and in the monosaccharides takes place mainly in the parts above the parasite (Haupt, 1994).

From the results of the translocation experiments with $^{14}CO_2$ and from the uptake studies we can conclude that sucrose is the preferable substance for the transfer of sugars from the host sieve tube to the parasite. Nevertheless, also the monosaccharide, glucose, that naturally occurs in the common apoplast between host and parasite could be, more or less, absorbed.

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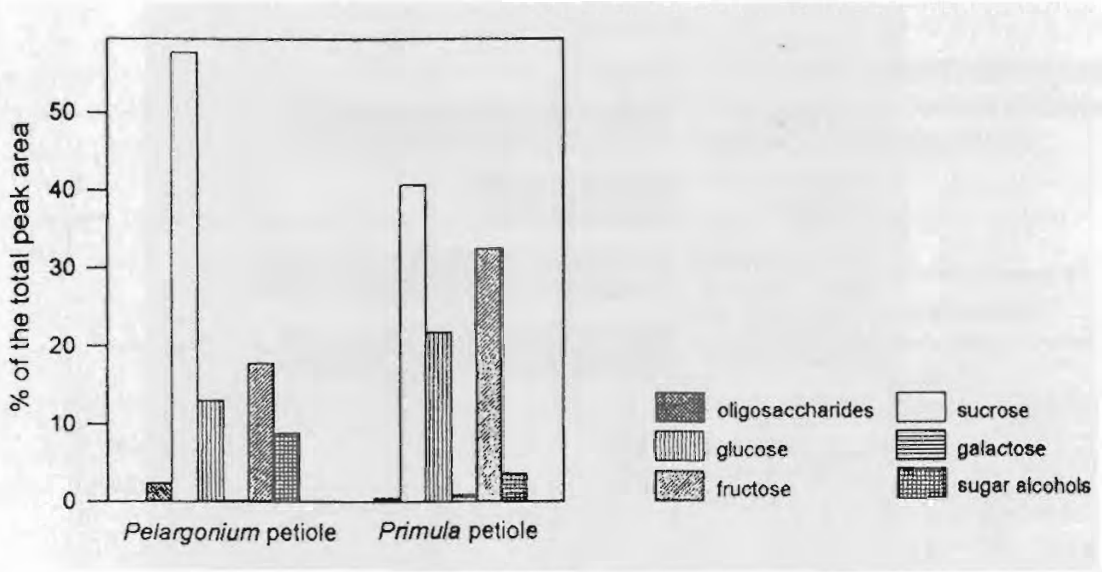

Figure 1

Sugar spectra of the nonparasitised petioles of the host plants *Pelargonium zonale* and *Primula obconica*. The content of sugar was determined by HPLC (n = 10).

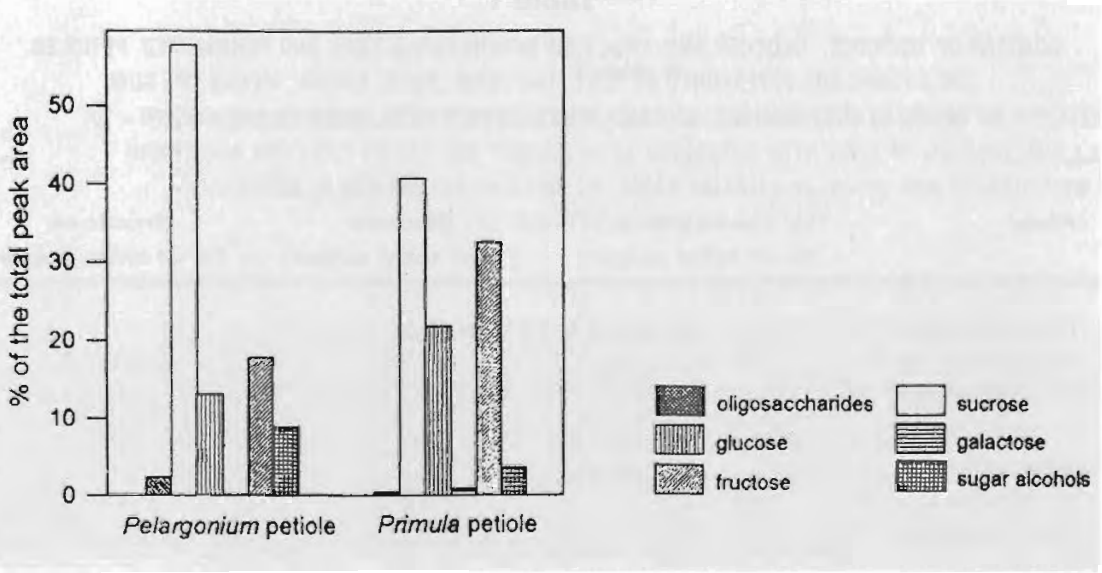

Figure 2

Ratio of sucrose to monosaccharides in different parts of the host-parasite-system *Pelargonium zonale-Cuscuta reflexa*. The content of sugar was determined by HPLC (% of the total sugars) and by the radioactivity measurement of the chromatographically separated methanol extracts (% of labelled sugars), respectively. $^{14}CO_2$ was applied to the host leaf for 5 min., the translocation duration was 3h (n = 10).

hange. This result shows that the rudimentary oot of dodder seedling is able to absorb mineral utrients for a few days after germination. A similar esult had previously been found concerning hosphate uptake (Fer, 1976).

aking into account the distribution of ^{35}S along he seedling, it is clear that a noticeable part of the ^{35}S absorbed into the root is then transported to he stem, since the ^{35}S content, expressed on a resh weight basis, is much higher in the stem than n the root (Table 1). Moreover about 15% of the bsorbed sulfate was reduced and is incorporated nto organic compounds (Table 2). Most of ^{35}S ontaining metabolites is present as cysteine Fig. 1). The other labelled substances occurring n the chromatogram have not been clearly dentified; among them minor amino acids such as ystathionine and homocysteine may occur. abelled compounds showing low hromatographic mobility seem to correspond to roducts deriving from oxidation of sulfur ontaining amino acids and glutathione. However ve can note that methionine could not be detected n the radiochromatogram. It may be that this mino acid is more sensitive to oxidation than ystein or that it is present at a much lower oncentration. Nevertheless an indirect proof that ethionine does occur in the seedling, exists since his amino-acid is the precursor of ethylene and olyamines (Zehhar and Fer, 1996).

rom the above it is clear that the dodder seedling s able to achieve sulfate reduction. The reduction rocess occurs with the same intensity in light 100 μmoles.m^{-2}.s^{-1}) or in darkness. So it may be oncluded that sulfate reduction in *Cuscuta* is not ght-dependent and that the reducing power is not rovided by photosynthesis.

2. Sulfur nutrition of the attached parasite

) Transfer of 35S from the host to the attached arasite. A mature leaf of a *Phaseolus aureus* lant parasitized by *C. lupuliformis* was supplied with $^{35}SO_4^{2-}$. Two days after foliar application, ^{35}S had been transported to the different sinks of the host-parasite system (Fig.2). Measurements of the radioactivity occurring in the different parts of the host-parasite system presented in Fig. 2 have given quantitative data on the distribution of ^{35}S . The host leaf (F2) supplied with $^{35}SO_4^{2-}$ contained 67% of the absorbed ^{35}S . The young expanding leaf (F3) and the apical bud (BA) of the host together accounted for 10.6% of the absorbed ^{35}S. Host roots and stem (including dodder hautoria) contained 7% of the absorbed ^{35}S. Finally the dodder shoot had imported 15.1% of the ^{35}S absorbed by the host leaf. So it appears that the dodder shoot had taken up about 50% of the ^{35}S exported out of the fed host leaf. This result clearly shows that dodder is the strongest sink for sulfur compounds exported out of the host source leaf, probably *via* the phloem pathway. Moreover it is clear that along the dodder shoot ^{35}S is mainly accumulated in the youngest part and in apical (A) and axillary (Ba) buds, *i.e.* in the growing regions where protein synthesis rate is very high.

Another experiment, performed using isolated *Pelargonium* leaves parasitized by *C. reflexa*, clearly shows that the dodder takes up S-containing compounds from the host phloem (Table 3). Indeed, in such a model, ^{35}S imported from the host leaf blade can move only by the phloem pathway (Fer, 1981; Fer and Chamel, 1983). This experiment confirms that dodder derives S-containing compounds from the host phloem.

An analysis of the ^{35}S-containing compounds found in the Pelargonium leaf and in C.reflexa was performed 24 hours after supplying host leaf blade with $^{35}SO_4^{2-}$. The results (Table 4) show that $^{35}SO_4^{2-}$ is the main labelled fraction of sulfur containing substances in both host and parasite. Nevertheless, labelled organic compounds expressed as percent of total ^{35}S, are much more abundant in parasite than in host tissues; this is particularly true for insoluble proteins and soluble metabolites.

From this experiment it is not possible to know the nature of the S-containing substances that are

transferred from host to parasite. However we suggest that it is $^{35}SO_4^{2-}$ which is taken up by the parasite and that inorganic sulfate is metabolized to a higher rate in dodder tissues than in the host.

b) Sulfate assimilation by isolated tissues of the parasite in vitro. To verify that tissues of the parasite do have the ability to reduce sulfate, slices cut in the stem of *C. reflexa* growing on Pelargonium were incubated for 12 hours into a $^{35}SO_4^{2-}$ solution, in the dark.

This experiment clearly shows that isolated tissues of the parasite reduce inorganic sulfate (Table 5). Moreover, the amounts of compounds containing reduced sulfur, expressed as percent of total ^{35}S, exhibit values very similar to those found in the tissues of the attached parasite after feeding on host source leaf with $^{35}SO_4^{2-}$ (Table 4). Here also cystein is the most heavily labelled metabolite (Fig. 3). So it can be concluded that dodder, in the parasitic stage, obtains sulfur mainly as inorganic sulfate from host phloem.

This result demonstrates that in autotrophic host plants such as *Pelargonium zonale* and *Phaseolus aureus*, sulfur taken up by the root and transported *via* the transpiration stream to mature leaves is then mainly distributed to sink organs *via* the phloem pathway as sulfate. This provides additional proof that inorganic sulfate is the main phloem-mobile form of sulfur in non-parasitic higher plants, a question which has been actively debated (Anderson, 1980 and 1990).

In dodder stem tissues where photosynthetic activity is very low (Fer, 1977), sulfate reduction is achieved and is not light-dependent. This is an important result since sulfate reduction in non-parasitic higher plants has always been considered to occur mainly in photosynthetic tissues of the leaf and to be light-dependent (Cram, 1990).

In conclusion, our results, as a whole, provide experimental evidence that dodder is not dependent on its host for reduced sulfur supply.

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Table 4

DISTRIBUTION OF ^{35}S AMONG THE MAIN S-CONTAINING COMPOUNDS IN THE *PELARGONIUM* LEAF BLADE AND IN THE ATTACHED *C. REFLEXA* SHOOT, 24 HOURS AFTER APPLICATION OF $^{35}SO_4^{2-}$ TO THE HOST LEAF BLADE. *VALUES ARE MEAN OF MEASUREMENTS PERFORMED SEPARATELY ON 3 PARASITIZED HOST LEAVES ± SE.

	Radioactivity expressed as 10^3. dpm or as % of total ^{35}S in each organ*			
Nature of S-containing compounds	Pelargnium leaf blade 10^3. dpm	(%)	*C. reflexa* shoot 10^3. dpm	(%)
insoluble compounds (proteins)	61.5 ± 7.0	3.63 ± 1	78.5 ± 8.1	13.2 ± 1.5
soluble polypeptides	44.5 ± 5.12	.62 ± 0.8	23.0 ± 1.8	3.88 ± 0.31
soluble metabolites (amino-acids)	42.7 ± 3.8	2.52 ± 0.6	70.3 ± 6.8	11.86 ± 1.3
inorganic sulfate (SO_4^{2-})	1547.0± 100.0	91.23 ± 6.1	421.0 ± 35.0	71.02 ± 6. 9

Table 5

DISTRIBUTION OF 35S INTO THE DIFFERENT S-CONTAINING COMPOUNDS IN ISOLATED *C. REFLEXA* STEM TISSUES INCUBATED INTO A LABELLED $^{35}SO_4^{2-}$ SOLUTION (1 μCI.ML^{-1}) FOR 12 HOURS. *VALUES ARE MEAN OF 3 MEASUREMENTS ± SE.

	^{35}S content *	
Nature of S-containing compounds	10^3. dpm.g^{-1} F.W	% of total ^{35}S
Insoluble compounds (proteins..)	494.0 ± 51.0	12.2 ± 1
Soluble polypeptides	382.0 ± 36.0	9.4 ± 0.8
Soluble metabolites (amino-acids...)	375.0 ± 38.0	9.2 ± 1
Inorganic sulfate (SO_4^{-2})	2800.0± 240.0	69.2 ± 6

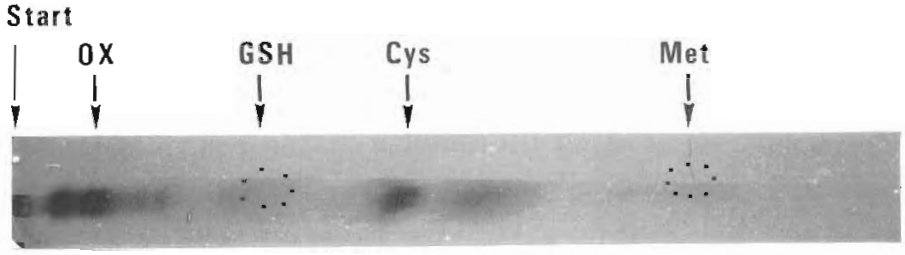


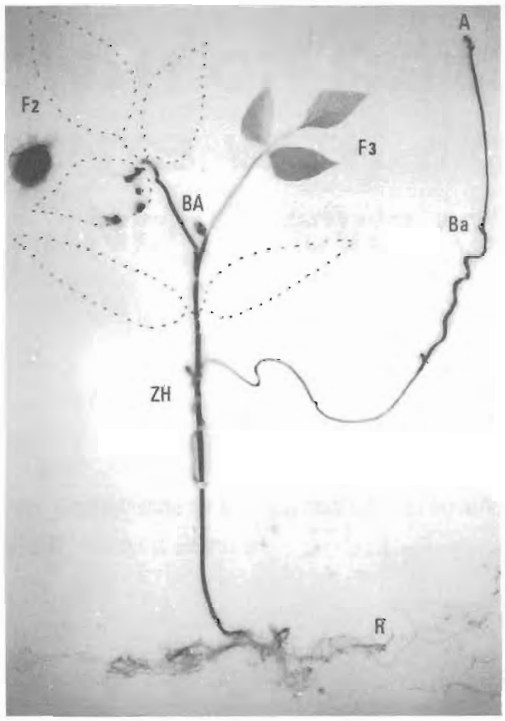
Figure 1

Radiochromatogram of the 35S-containing soluble metabolites extracted from *C. lupuliformis* seedlings grown for 7 days after sowing on diluted knop solution labelled with $^{35}SO_4^{2-}$ (1 μCi.ml^{-1}).
Arrows indicate the position of the main S-compounds on the chromatogram, as determined by chemical standards (Cys: cysteine ; Met : methionine; GSH : glutathione; OX: products deriving from oxidation of Cys and GSH).

Figure 2

Autoradiography of a Phaseolus aureus plant parasitized by *C. lupuliformis*, two days after feeding a mature leaf of the host (F2) with $^{35}SO_4^{2-}$. The host leaf disc where $^{35}SO_4^{2-}$ had been applied (arrow) was cut off at the end of the experiment and washed with water to discard unabsorbed labelled sulfate.

F3 : young expanding host leaf
BA : Apical bud of the host
ZH . part of the host stem where dodder haustoria are attached
R : host roots
A : Apical bud of Cuscuta shoot
Ba . Axillary bud of Cuscuta shoot



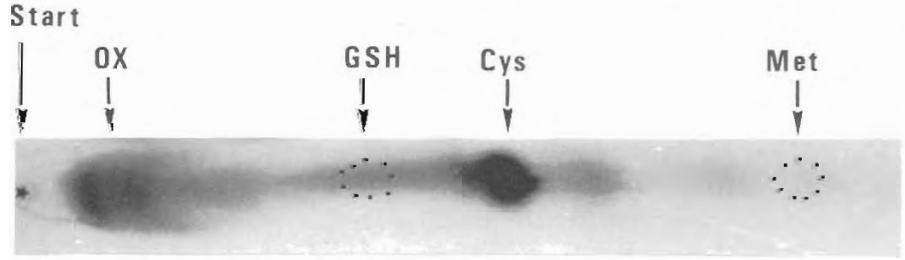


Figure 3

Radiochromatogram of the ^{35}S-containing soluble metabolites extracted from isolated *C. reflexa* stem tissues incubated in a $^{35}SO_4^{2-}$ solution (1 μCi.ml^{-1}) for 12 hours - (Cys., Met., GSH and OX, see legend of Fig. 1).

Introduction

Recently the relative dependence of the angiosperm parasite *Cuscuta reflexa* Roxb. on nutrition from the phloem and the xylem of its host *Lupinus albus* (white lupin) has been determined and the proportion via the phloem varied from 99.5% for carbon, 93.6% for nitrogen, 74% for potassium to close to 0% for calcium (Jeschke et al., 1995), the remainder being supplied by the xylem. When parasitizing this host, *C. reflexa* formed an overriding sink, attracting 56% of the current photosynthate and all of the fixed N and also a more than equivalent (123%) amount of N mobilised from N reserves previously accumulated by the host. As a result of this sink activity the net photosynthesis of the host appeared to be stimulated whilst nitrogen fixation was severely inhibited (Jeschke et al., 1994). Considering the enormous sink strength of *C. reflexa* the question arises whether the supply of either carbon or nitrogen limits growth of the parasite. In the case of suboptimal supply of N and excessive availability of C, *Cuscuta* in principle is able to excrete sucrose from extrafloral nectaries (Schaffner, 1979), however, it is not known whether this is a means of regulating the relative supply of C and N in this parasite. Interestingly secretion was high when *Cuscuta* parasitized *L. albus* but negligible when *Coleus* was the host.

In order to study possible growth limitations *C. reflexa* has been grown on *Ricinus communis* (castor bean) plants fed with varied N-supply. The growth of host and parasite, nitrogen concentrations, secretion of sucrose and the photosynthesis of the host were measured.

Material and Methods

Ricinus communis was grown on quartz sand, as detailed by Jeschke and Wolf (1988) and one third each of the plants was watered daily with a nutrient solution containing either 0.2, 1 or 5 mM nitrate. thirty five days after sowing (DAS) shoot tips of *C. reflexa* were allowed to attach to the lower stem of the host plants. Successful haustoria were formed within 6 days; plants were harvested 24 or 32 days after establishing the parasite. Host plants were divided into the individual leaves, stem parts and roots, the parasites into the haustorial region, shoot axes and the 5-10 cm apical tip region, the samples were weighed, freeze dried and analyzed for C and N in a CHN-Analyzer (Heräus, Hanau, Germany). Photosynthesis was measured using a LCA 2 portable IR analyzer (ADC Instruments, Hoddesdon, UK). Sucrose secretion was measured by washing a known surface area of *Cuscuta* with deionized water and determining the sugar by refractometry.

Results

Dry weight development and shoot growth

Dry weights of shoot and the whole plant of *Ricinus* decreased almost linearly with lower N supply (Fig. 1), while the root to shoot ratio increased. When parasitized by *Cuscuta* shoot and particularly root growth (see the difference between plant and shoot in Fig. 1) were substantially depressed at each level of N supply, but the decrease was somewhat larger under limiting N supply: Shoot dry weights were decreased by 30, 35 or 43% in the presence of *Cuscuta* at 5, 1 or 0.2 mM nitrate respectively. The growth of *Cuscuta* was strikingly depressed by limiting N supply to a similar degree as was the host, so that the sum of dry weights of parasite and host closely resembled that of the non-infested *Ricinus*. The dramatic decrease in growth of *C. reflexa* on N-limited hosts is also seen from the development of the stem length (Fig. 2). While in 29 days the parasite reached a total length of 40 m on *Ricinus* fed 5 mM nitrate, it was merely 13 or 3.5 m on hosts supplied with 1 or 0.2 mM nitrate.

Nitrogen concentrations and contents

N levels in shoot and root tissues of control *Ricinus* plants decreased strongly with lowered

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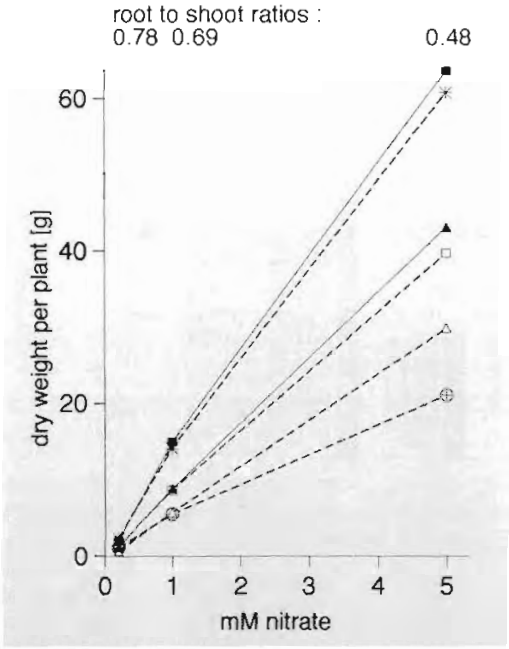

Figure 1

Dry weights of control plants of *Ricinus* (——■—— total plant, ---▲--- shoot) and of *Cuscuta*- infected plants (---□--- total host, ---△--- host shoot, ---⊕--- *Cuscuta*, ---✱---sum of Cuscuta plus host) at varied nitrate nutrition of the host. Harvests 59 days after sowing and 24 days after infection with *Cuscuta*. Above the Figure the root to shoot ratios of uninfested *Ricinus* are given.

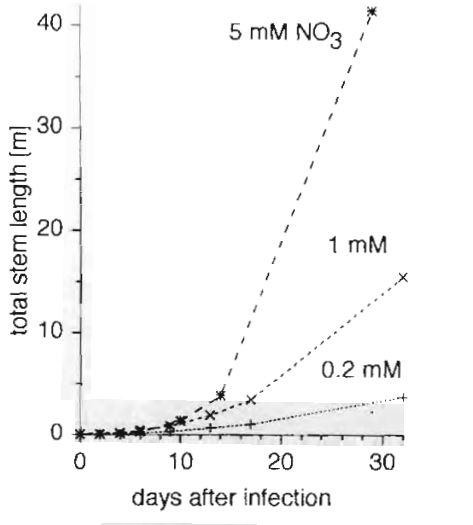

Figure 2

Development of the total shoot length of *Cuscuta reflexa* depending on the nitrate nutrition of the host.

III.24

ROLE OF ETHYLENE AND POLYAMINES IN THE CONTROL OF GROWTH AND DEVELOPMENT OF *Cuscuta lupuliformis* SEEDLINGS

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ABSTRACT

Dodder seedlings exhibit a very high growth rate allowing them to attach to a host plant. High growth rates of seedling stems and the occurrence of a hook are common features also found in etiolated autotrophic plants where ethylene is known to play an important role. This strongly suggests that ethylene may control the growth of the dodder seedling stem. Since ethylene and polyamines share a common precursor (S. adenosyl methionine) the possible role of ethylene and polyamines in the control of dodder seedling growth was investigated.

Ethylene production is much higher in the stem than in the root, the highest value being measured in the apical part of the dodder seedling stem including the hook. During seedling stem growth the highest ethylene production coincides with the lowest growth rate. So ethylene negatively controls the growth of dodder seedling stem. This effect of ethylene was confirmed by using inhibitors of ethylene synthesis or action. Chloroethyl phosphonic acid and 2.4 D both inhibit the growth of seedling stem by releasing ethylene *in vivo* and stimulating endogenous ethylene production respectively.

Regarding polyamine, it was shown that spermidine and spermine concentrations were highest in the upper part of the stem, while putrescine level was highest in the lower part of the stem. Hence spermidine and spermine seem to be involved in the control of cell division while putrescine is implicated in the control of cell elongation.

These results, as a whole, strongly suggest that the growth rate of the dodder seedling stem is controlled by the balance Ethylene / polyamines. This work may lead to new strategies for dodder control.

Additional key words : Parasitic angiosperms, cell division, cell elongation, hormonal control.

Both ethylene production and seedling growth were measured in the presence and in the absence (control) of each effector. All the inhibitors of ethylene synthesis induced a decrease in ethylene production and a strong stimulation of growth (Table 1). Silver thiosulfate, an inhibitor of ethylene action, which is thought to block the receptor of the gaseous hormone (Beyer,1979), strongly enhanced both ethylene production and seedling growth ; this result suggests that ethylene controls its own production, probably by a feedback mechanism. Chloroethylphosphonic acid, by releasing ethylene *in vivo*, strongly reduced seedling growth. Moreover 2,4-D, a xenobiotic substance exhibiting auxinic action, stimulated ethylene production and strongly inhibited the growth of the seedling stem. Inhibitors of ethylene synthesis or action had no effect on the growth rate of the seedling root. Ethylene produced by the seedling controls only the growth of the seedling stem.

Such an action of ethylene in lowering the growth rate of etiolated seedling stems in the early stage of development has been reported in different plant materials (Perez-Gilabert *et al.*, 1991). The effect of 2,4 D observed in our experiment suggests that ethylene production in *C. lupuliformis* seedlings is controlled by endogenous auxin synthesized in the stem apex. However, it must be emphasized that the growth rate of the seedling stem, even if it is reduced by ethylene in the early stage of development, exhibits a very high value. This may perhaps be explained by the fact that the stem meristem of the seedling is not restricted to the apex where rudimentary minute leaves are initiated but extends approximately 6 mm downwards and that, beyond this 6 mm region, cell elongation gradually replaces cell division (Agaev *et al.*, 1988). Among polyamines, spermidine and spermine are known to be necessary for cell division and putrescine to be involved in cell elongation (Flores *et al.*, 1989). Moreover polyamines and ethylene share the same precursor (S-adenosyl methionine). Consequently, we decided to investigate the possible role of polyamines, beside that of ethylene, in the control of dodder seedling growth.

Contents in polyamines during seedling growth

Putrescine (Put), spermidine (Spd) and spermine (Spm), present in the dry seed, are also synthesized during seedling growth as shown using ^{14}C-Putrescine (results not presented). Contents in polyamines in the entire seedling showed important changes depending on the growth stage considered. The highest Put content was detected in 5 day-old seedlings, when the growth rate is high and ethylene production is decreasing (Table 2). The highest Spd and Spm contents were measured in 2 day-old seedlings, when ethylene production rate was the highest. These results suggest that in *C. lupuliformis* seedlings, the growth rate of the stem may be under control of both ethylene and polyamines. To further investigate this hypothesis, polyamines and ethylene production were measured in the different parts of seedling.

Ethylene production and content in polyamines in the different parts of the seedling

Six day-old control seedlings were divided into 3 segments :

- upper part of the stem (1.2 cm long), including the "giant" shoot meristem
- lower part of the stem (2 cm long), including the region where cell elongation occurs.
- Fully-developed rudimentary root (2 cm long)

Analyses were performed separately on each kind of segment. The results clearly show the occurrence of a pronounced gradient of ethylene production along the seedling (Table 3). On a fresh weight basis, ethylene production is about 30 times higher in the apical part of the stem than in the root. Contents in Spd and Spm show a similar distribution gradient, although much less marked than that of ethylene. In the case of Put, the highest content was measured in

the lower part of the stem. Consequently, it is clear that the highest ethylene production and Spd and Spm contents occur in the upper part of the stem exhibiting high cell division activity. On the other hand, the highest Put content is observed in the lower part of the stem where mainly cell elongation occurs.

Conclusion

Ethylene negatively controls the growth of the *Cuscuta* seedling stem as is clearly shown by measuring endogenous ethylene production and by application of several effectors acting on production or action of the gaseous hormone. The very high ethylene production rate occurring in the apical part of the seedling stem might inhibit cell elongation, a well-known action of the hormone. In the same stem part, the high contents in Spd and Spm might stimulate cell division. Moreover it could be suggested that the high Spd content is responsible for the stimulation of ethylene synthesis, but this hypothesis needs to be verified. It seems that the additive effects of ethylene Spd and Spm in the stem tip may explain the intensive cell division activity which is necessary to sustain the high growth rate observed in the stem.

In the lower part of the stem, the high level of Put seems to be partly responsible for the cell elongation that occurs in this region. Endogenous auxin exported from the stem apex probably also contributes to the control of cell elongation.

Ethylene and polyamines are clearly involved in the control of dodder seedling stem growth, but the respective action of these substances and their interaction needs to be further investigated. Nevertheless on the basis of the results presented here it is possible to envisage the use of auxinic herbicides or ethylene releasing agents such as ethephon to lower *Cuscuta* attack in several crops.

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Table 2

VARIATIONS OF POLYAMINE CONTENTS IN THE ENTIRE SEEDLINGS AT THE DIFFERENT GROWTH STAGES

Seedling age (day after sowing)	Polyamine contents (n.moles.g^{-1}. F.W.) Put	Spd	Spm
2	51 ± 5.5	62 ± 5	11 ± 4
3	84 ± 27	126 ± 30	24 ± 8
4	88 ± 11	88 ± 13	11 ± 2
5	94 ± 29	87 ± 19	13 ± 3
6	73 ± 17	71 ± 15	15 ± 4
7	65 ± 14	57 ± 10	15 ± 6
8	57 ± 20	40 ± 12	11 ± 3

Data correspond to mean value of three replicates ± S.E.

Table 3

ETHYLENE PRODUCTION AND POLYAMINE CONTENTS IN THE DIFFERENT PARTS OF 6 DAY-OLD CONTROL SEEDLINGS

Seedling part	Fresh weigh mg	Ethylene production p.moles.g^{-1}. F.W.	Polyamine contents (n.moles.g^{-1}. F.W.) PUT	Spd	Sp
upper part of the stem (1.2 cm lenght)	3.3 ± 0.3	212,8 ± 62	178 ± 7	373 ± 19	110 ± 18
lower part of the stem (2cm lenght)	5.5 ± 0.6	80.6 ± 25	189 ± 30	149 ± 27	21 ± 4
Root (2cm lenght)	18.6 ± 1.6	6.3 ± 0,4	52 ± 17	21 ± 9	— ND

— ND (not detected).

Data correspond to mean value of three replicates ± S.E.

III.25

EFFECT OF DODDER *(Cuscuta chinensis)* ON THE PRODUCTIVITY OF SOME VARIETIES OF ALFALFA *(Medicago sativa)*

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ABSTRACT

Dodder (*Cuscuta chinensis*) infection on alfalfa was gradually increased during the first three summer months of the growing season, then decreased duringthe winter season. Dodder infection was higher in plots sown at 40 cm row spacing than in plots sown in 20 cm rows. Spring field variety was the most tolerant among the tested varieties, followed by C.U.F. 101 and then Si river varieties. Dodder infection significantly reduced green alfalfa fodder in summer cuts. The overall reduction in the first three cuts was 26.45 %. Alfalfa seed yield obtained from the infested plots and their germination percentage were significantly reduced by 51.0% and 52.6% respectively.

IV.1

EFFECTS OF SOIL SAUNA ON *Orobanche* SEEDS

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ABSTRACT

Effects were studied of soil animals on dispersal and germination of *Orobanche* seeds. The tests included four *Orobanche* species, *O. aegyptiaca*, *O. oxyloba*, *O. crenata* and *O. minor*. Effects of springtails (Collembola), potworms (Enchytraeidae) and earthworms (Lumbricidae) were studied. Seeds were presented on filter paper; these were dispersed and eaten by pot- and earthworms; *O. crenata* was eaten last and least. No quantitative data were collected for survival after passage through the animals' digestive tracks, but a fair number appeared to have passed earthworms without damage; the germinability of the latter was only slightly reduced.

In a pot experiment, using tomatoes as host plants, seeds of *O. aegyptiaca* and *O. oxyloba* were put on the soil surface, and springtails, potworms or earthworms added. At harvest, the vertical distribution of seeds was determined and the broomrape attachments recorded per layer. Earthworms especially affected both numbers and depth distribution. They brought the seeds down to 12 cm, which was the depth of the pots. Total numbers of attachments per pot were higher than in the control without animals. The attachments were sometimes close together in small clumps, probably due to the deposition of casts containing several seeds.

Keywords: Earthworms, *Orobanche*, seed dispersal, germination.

replicates of each treatment were placed in a random block design in a greenhouse at 18/15 °C and 12 h daylength. Seed transport was established in two ways. First, four vertical 2.2 cm diameter soil samples were taken per pot and divided into 2 cm layers. The four samples per layer were combined, and the number of seeds per layer determined after flotation in a concentrated $CaCl_2$ solution (1140 g $CaCl_2$.2 H_2O/l). Next, the pot contents were cut into 2 cm layers, the roots washed clean, and the broomrape attachments counted per layer.

Finally the numbers and distribution of young broomrapes were recorded in some 6 cm wide and 13 cm deep soil cores taken in an *O. minor* population in a grassland in S.Limburg. Eight samples were taken in November 1994, and 8 in april 1995. These cores were cut into 2.5 cm layers before counting.

Results

The feeding experiment showed that the springtails were too small to have any effect on the seeds. Potworms ate the seeds, which could be observed in the animal gut, measuring over half of the size of the animals diameter. The seeds had a normal appearance when excreted. Since it was impossible to distinguish seeds which had been eaten and those which had not, the effect on germinability could not be established.

Earthworms were actively feeding on the seeds; since no alternative food was offered, the casts almost exclusively consisted of broomrape seeds, sometimes mixed with some filter paper. Moreover the seeds were spread all over the inner sides of the containers in which the earthworms were kept; these seeds were transported on the rather sticky skins of the animals. Like potworms, earthworms started ingesting seeds of *O. aegyptiaca*, whereas *O. crenata* was taken last and least. The proportion of seeds lost by consumption by earthworms could not be established. Seeds in the casts had a normal appearance; the tests showed that gut passage had either none or a negative effect on their germinability (Fig.1, right). In the controls with water only, a stimulating effect of gut passage was found in one case, whereas there was no effect or a negative response in all others (Fig.1, left). There were no consistent differences between broomrape species.

Data on vertical transport of seeds in pot soil, as indicated by numbers of seeds per soil layer as well as numbers of established broomrapes, are given in Fig.2. Although numbers still were highest in the top layer, earthworms had clearly affected seed distribution, in contrast to springtails and potworms, where the depth distribution did not differ significantly from the control. The data of the pots without animals point to a certain degree of 'natural' transport, up to a few centimeters' depth. Some scattered broomrape attachments were observed in pots where no seeds had been added.

Figure 3, finally, shows the depth distribution of *O. minor* under natural grassland conditions. The majority of attachments were found in the litter layer and a few centimeters below, *i.e.* generally slightly deeper than in the pot experiment.

Discussion

The effects of soil fauna clearly differ between the species: *Folsomia candida* hardly has any influence, since it is too small to really affect the seeds. The slightly larger potworms may have an effect, but this needs further study, since seeds that had been eaten and those which had not, could not be distinguished.

Like in other plant species, effects of earthworms on seeds appeared to be manyfold (Bolton and Phillipson, 1976; Shumway and Koide, 1994; Willems and Huysman, 1994). They handled the broomrape seeds, which got attached to their skins and could be transported over appreciable distances in the containers. Moreover, the earthworms ate the seeds, and large numbers passed the digestive tract; they appeared to fit in the seed size classes that are preferred by earthworms (Shumway and Koide, 1993).

Apparently a large proportion are able to withstand gut conditions, and remain germinable, as was also reported by Jacobsohn *et al.* (1987), who introduced broomrape seeds into the rumen of sheep. These authors suggested that survival was lower in *O. crenata* than in *O. aegyptiaca* and *O. cernua*. Therefore, animal consumption might account for part of the c.50% losses between production and germination of seeds of *O. crenata* reported by López-Granados and García-Torres (1993). We noticed some slight differences between *Orobanche* species, where *O. aegyptiaca* was found to be handled more, and *O. crenata* appeared to be eaten last and least. The latter species seems to have components in its seed coat that reduce attack by soil organisms. Effects on germinability did not differ between species.

A stimulating effect of gut passage on germination was noticed in one case. However, a definite conclusion is impossible, since we could not distinguish the effects of stimulus and reduction. If a stimulus does occur, it will reduce population numbers under field conditions, since the chance that seeds are deposited close to roots of a host is relatively small, especially if it concerns host species in a mixed vegetation as in the case of *O. minor*.

Passage of seeds through the earthworm gut takes a couple of hours (Bolton and Phillipson, 1976). During this period the animals move around, and thus transport the seeds before deposition (Willems and Huysman, 1994). The effects were clear in the pot experiment, where the seeds were found at the bottom of the pots. In some cases attachments were found in small clumps, probably a result of the concentrated seed deposition in worm casts. *Orobanche* attachments in pots where no seeds had been added may have resulted from earthworms passing between pots during the experimental period, indicating that earthworms may transport seeds over a couple of meters' distance. Our data, like those presented by Jacobsohn *et al.* (1987) and Berner *et al.*(1994) indicate that appreciable numbers of seeds are able to survive consumption. Transport then will depend on the distance covered by the consumer during the time between eating and deposition of seeds in feces. This may vary from a few centimeters in small, little active animals, up to several kilometers for seeds which are passing through large herbivores, birds, etc.

Comparison of figures 2 and 3 indicates that the pattern of seed distribution under natural conditions somewhat differed from the pattern in the pots, with relatively more attachments in deeper layers. This may be a result of a longer period of dispersal.

Numbers of established plants were highest in pots with earthworms. This could be due to a range of factors. First, conditions for host root growth and seed germination (oxygen, nitrogen-content, moisture, etc.) may be better, due to the tunnels made by the earthworms. Next, according to Cortez and Bouché (1992) earthworms may eat live roots, resulting in more branched roots, *i.e.* more sites of attachment for broomrapes. Finally, the number of nearby broomrape individuals was lower at lower positions, which may have reduced mortality due to competition.

In concluding it can be stated that soil fauna, especially earthworms, do affect broomrape seeds in various ways. Seeds are eaten, and during digestion may be transported, both downwards (Fig. 2) and upwards (Willems and Huysman, 1994), and in a horizontal direction. Although seed numbers may be reduced to some extent, large amounts appear to survive; *O. crenata* seems to be the most resistant of the species tested. More quantitative data are required to include these effects in population models and establish their significance under various field conditions.

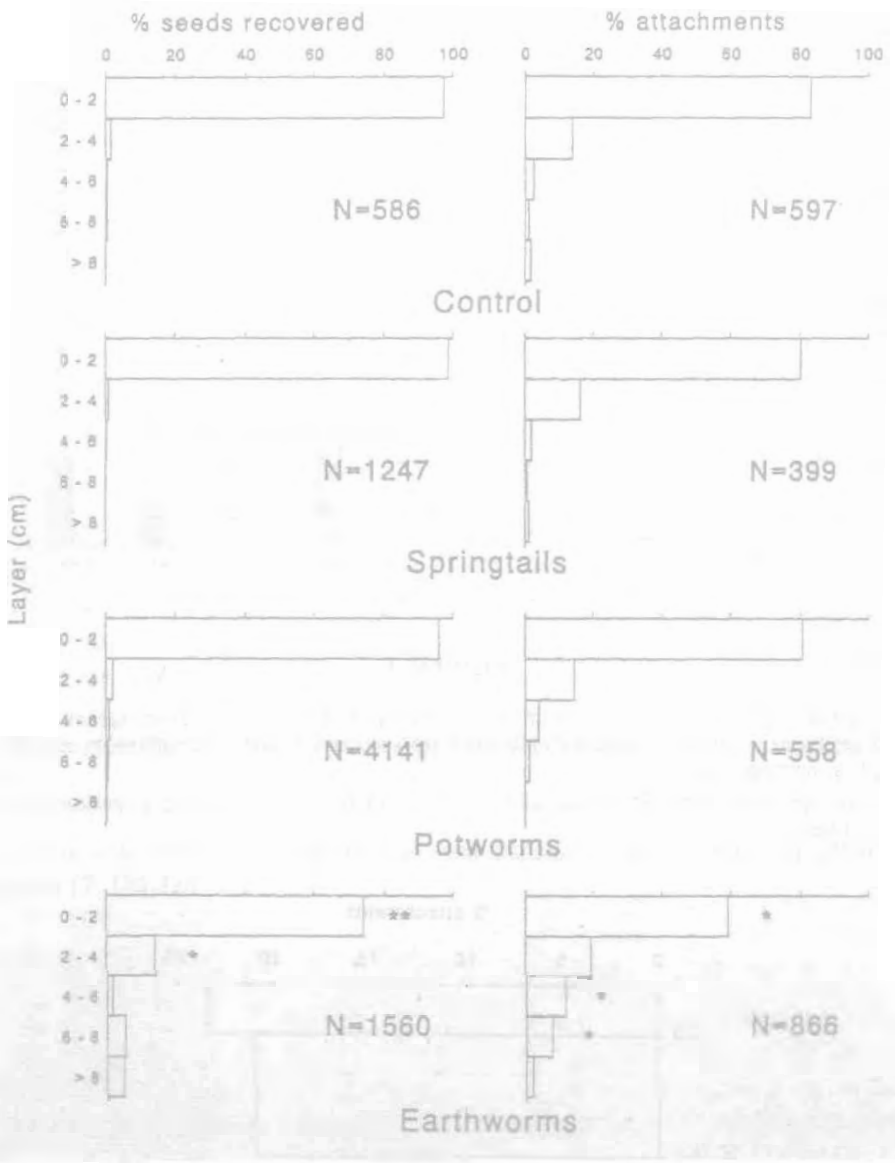

Figure 3

Depth distribution of attachments of *O. minor* on *Trifolium repens* in chalk grassland. Percentages per 1 cm layer of total number collected.

IV.2

Environmental Influences on Germination of *Orobanche*

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ABSTRACT

In vitro germination studies were conducted to study the effect of nitrogen-containing nutrients, osmotic potential, and temperature on germination and radicle elongation of *Orobanche* spp. Radicle lengths of both *O. aegyptiaca* and *O. ramosa* were inhibited to a greater extent by NH_4NO_3 and NH_4Cl than by KNO_3, but the species differed in sensitivity to the nutrients. *O. ramosa* was more sensitive than *O. aegyptiaca* to inhibition by all concentrations of NH_4Cl and NH_4NO_3. Osmotic potential influenced *Orobanche* germination and radicle elongation; however, no correlation was found between osmotic potential and inhibition by nitrogen-containing solutions. Therefore, inhibition of germination and radicle length by NH_4^+ is not due to simple osmotic effects. *Orobanche* species had different temperature optima for germination, but similar temperature optima for maximum radicle elongation (23 to 25 C).

Additional key words: *O. cernua, O. crenata.*

Effect of osmotic potential

Seeds of *O. aegyptiaca*, *O. cernua* Loefl., *O. crenata*, and *O. ramosa* were surface cleaned as outlined above. Various levels of osmotic potential (0 to -0.8 MPa) were induced on preconditioned *Orobanche* seeds with analytical grade polyethylene glycol 8000 (PEG). PEG solutions were replaced every 2 days with fresh solutions to maintain precise osmotic potentials. All the treatments contained GR-24 at 10 mg/L. Osmotic potential measurements of PEG and nutrient solutions were taken with a vapor-pressure osmometer (Wescor Inc., UT). GFFP sandwiches were incubated at 25 C and germination and radicle length measurements were taken 8 days after incubation.

Effect of temperature

Seeds of *Orobanche* (cleaned and handled as previously described) were preconditioned and germinated under eight different temperatures, ranging from 16 to 32 C. Preconditioned seeds were exposed to 10 mg/L GR-24 and germination and radicle length measured 8 days later.

All experiments were conducted in a randomized complete block design with four replications. Treatment means in the nutrient and temperature experiment were compared by ANOVA. FisherÕs Protected Least Significant Difference (LSD) Procedure at 5% level of significance was used to compare the treatment means in the osmotic potential experiment.

Results

None of the three nitrogen-containing nutrients influenced the germination percentage of *O. aegyptiaca* (Figure 1a); however, both NH_4Cl and NH_4NO_3 significantly decreased the germination percentage of *O. ramosa* (Figure 1b). In the presence of 25 mM NH_4NO_3, germination was reduced by nearly 50% compared to the 0 mM concentration. The nitrogen source significantly affected radicle length of both species (Figure 1c and 1d). Both ammonium salts decreased the radicle length of the two species compared to KNO_3. *Orobanche ramosa* was again more sensitive than *O. aegyptiaca* to inhibition by NH_4Cl and NH_4NO_3 as radicle length was decreased approximately 45% by 5 mM NH_4NO_3; whereas, 25 mM NH_4NO_3 was required to obtain the same level of inhibition in *O. aegyptiaca*.

The osmotic potential of the surrounding solution significantly affected the germination percentage of all species tested except *O. crenata* (Table 1). In general, germination percentage was highest for all species when osmotic potential was 0 to -0.2 MPa and gradually declined as osmotic potential decreased to -0.8 MPa (Table 1). Similarly, radicle length of all four species was significantly influenced by osmotic potential. The longest radicles were recorded at -0.15 MPa for all species and length decreased as osmotic potential either increased to 0 or decreased past -0.2 MPa.

Osmotic potential of the nutrient solutions was measured to relate influence of nutrient concentration to osmotic effects. Osmotic potential of all three nutrient solutions, KNO_3, NH_4NO_3, and NH_4Cl, were similar at 5 mM (-0.13 MPa), 10mM (-0.14 MPa), and 25 mM (-0.17 to 0.19 MPa) (Table 2).

Temperature significantly influenced germination percentage of all four species. Optimal temperatures for germination of *O. aegyptiaca*, *O. cernua*, *O. crenata*, and *O. ramosa* were approximately 25, 26, 20, and 28 C, respectively (Figure 2). The radicle length of all species was also significantly affected by temperature. All species showed greatest radicle elongation at 23 to 25 C except *O. ramosa* which had a broader optimal range extending up to 28 C. Radicle elongation of all species was dramatically reduced as temperature increased to 32 C.

Discussion

For both *O. aegyptiaca* and *O. ramosa*, NH_4NO_3 and NH_4Cl were more inhibitory compared to KNO_3 (Figure 1). Our results confirm previous reports of the ammonium form of nitrogen being more inhibitory to *Orobanche* than the nitrate form (Jain and Foy, 1987; Pieterse, 1991). However, there have been no previous reports of differential sensitivity to ammonium among *Orobanche* species. The observation that *O. ramosa* was more sensitive than *O. aegyptiaca* to ammonium-containing compounds indicates that there are inherent differences between these closely related species in response to ammonium. An understanding of the biological basis for these differences may provide insight into optimizing *Orobanche* growth inhibition by ammonium in the field. Furthermore, these differences in sensitivity among species caution against generalizing about the efficacy of *Orobanche* control by nitrogen fertilizers.

Osmotic stress was proposed to be a component of nitrogen-induced inhibition of *Orobanche* germination (Abu-Irmaileh, 1981). Our results agree with the observations of Linke (1987) that osmotic stress reduces *Orobanche* germination. However, even though 25 mM concentrations of NH_4NO_3 and NH_4Cl were highly inhibitory to radicle development of *Orobanche*, the osmotic potentials of these solutions were close to -0.15 MPa, which was the optimum value for both germination and radicle elongation. Thus, there appears to be no correlation between osmotic potential, per se, and ammonium induced inhibition.

The differences in temperature optimum for germination and radicle elongation reflect fundamental differences between these two aspects of development. Although different temperature requirements have been described for germination of *Orobanche* species (Sauerborn, 1991), the effect of temperature on radicle growth has not been addressed. Unlike germination, the optimal temperature for radicle growth was the same for all species (between 23 and 25 C). This suggests that radicle elongation is determined by the rate of metabolic activity and is similar for all species. These findings emphasize the uniqueness and sensitivity of the germination trigger. A better understanding of the influence of temperature on *Orobanche* development after germination may lead to improved timing of control measures.

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Table 1

EFFECT OF OSMOTIC POTENTIAL ON GERMINATION PERCENTAGE AND RADICLE LENGTH OF FOUR *OROBANCHE* SPP. OSMOTIC CONDITIONS WER GENERATED WITH PEG AND APPLIED TO PRECONDITIONED SEEDS

	GERMINATION %			
Osmotic potential (-MPa)	*O. aegytiaca*	*O. cernua*	*O. crenata*	*O. ramosa*
0	80	22	37	0
0,15	60	50	38	48
0,2	67	46	36	46
0,3	46	47	36	26
0,4	49	41	37	33
0,5	47	2-	32	27
0,6	47	20	24	23
0,8	0	10	21	5
lsd (0,05)	81	6	21	5
		Radicle length (nm)		
0	0,38	0,0	0,0	0
0,15	0,55	0,33	0,4	0,28
0,2	0,33	0,2	0,18	0,23
0,3	0,2	0,2	0,1	0,15
0,4	0,23	0,23	0,1	0,15
0,5	0,18	0,15	0,1	0,18
0,6	0,23	0,13	0,1	0,18
0,8	0	0,1	0,1	0,05
lsd (0,05)	0,13	0,11	0,1	0,1

Table 2

OSMOTIC POTENTIAL OF NUTRIENT SOLUTIONS USED IN NITROGEN-INDUCED INHIBITION EXPERIMENTS

	OSMOTIC POTENTIAL (-MPa)		
Concentration (mM)	KNO_3	NH_4NO_3	NH_4Cl
5	0,13	0,13	0,13
10	0,14	0,14	0,14
25	0,19	0,17	0,18

IV.3

BIOLOGICAL AND CHEMICAL INHIBITION OF *Orobanche* SEED GERMINATION

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ABSTRACT

Radicle diffusates of fenugreek (*Trigonella foenum graecum*), lupins (*Lupinus termis*), coriander (*Coriandrum satifum*) and turnip (*Brassica rapa*) were significantly reduced the stimulatory effect of GR_{24}. Results showed the possibility of using these crops in an intercropping system with faba bean and tomato to reduce the infection rate of *Orobanche* plants attached to their hosts. Hydrogen peroxide and sodium hypochlorite significantly decreased the germinatio of *Orobanche* seeds. Inhibitory effect increased by increasing the concentration of the tested solution or/and the duration of seed treatment. However, the wetable sulfur decreased the germination percentage of *Orobanche* seeds. Such reduction increased by increasing sulfur content in GR_{24} solution.

stimulated by GR_{24} alone (80.16% and 59.06% respectively) was significantly higher than these of seeds treated with GR_{24} mixed with radicle diffusates of turnip (TRD), fenugreek (FRD), coriander (CRD) and lupin (LRD). Germination percentage of *O. ramosa* seeds in Gr_{24} (2 ppm) mixed with TRD, FRD, CRD or LRD (1:1, v:v) were 4.79%, 56.75%, 65.10% and 57.77% respectively. Meanwhile the germination percentage of *O. crenata* seeds were 38.13%, 38.17%, 34.86% and 31.11% respectively in the above mentioned mixtures. The germination percentage of *O. ramosa* and *O. crenata* (2.75% & 1.42% in TRD, 2.22% & 0.25% in FRD, 5.00% & 1.22% in CRD and 5.15 & 0.0% in LRD) were significantly less than these of seeds treated with water alon (9.49 & 2.87 Respectively).

Data presented in Table 2 revealed that *O. ramosa* and *O. crenata* seeds did not germinate when treated with 30 V of H_2O_2 for 10, 20, or 30 minutes or with 15 V of H_2O_2 for 30 minutes. Insignificant higher germination (1.75%) was detected in *O. ramosa* seeds treated with 15 V H_2O_2 for 20 minutes. Germination percentage of *O. ramosa* and *O. crenata* seeds treated with 1 ppm GR_{24} (79.62% & 60.42% respectively) were significantly decreased when seeds previously treated with 10 V of H_2O_2 for 10 mins. (58.22% and 23.0% respectively), 20 minutes (40.25% and 20.3% respectively) and 30 mins. (36.6 & 19.6% respectively).

However that the germination percentages of *Orobanche* seeds significantly decreased by increasing either the concentration of NaOCl or the time of treatment. Seeds of *O. ramosa* and *O. crenata* untreated with NaOCl were germinated by 80.07% and 61.17% respectively on GR_{24} solution. Meanwhile germination percentages of seeds treated with 1.3% NaOCl for 5, 10, and 15 minutes were 66.07%, 57.05% and 41.95% respectively for *O. ramosa* and 46.25%, 30.87% and 27.00% respectively for *O. crenata*. The highest concentration of NaOCl (5.25%) completely inhibit the germination of *O. ramosa* and *O. crenata* seeds regardless treatment longevity. Germination of *O. crenata* seeds was also completely inhibited when seeds were treated for 10 and 15 minutes with 2.7% NaOCl solution. *O. crenata* seeds treated for 5 minutes with 2.7% NaOCl were germinate by 9.75%.

Concerning the effect of sulphur data in Table 2 revealed that the germination percentage of *O. ramosa* and *O. crenata* seeds treated with GR_{24} (79.87% and 60.30% respectively) were significantly higher than these of seeds treated with GR_{24} + 0.25 or 0.5 g/L of sulphur (59.00 % and 57.40% respectively for *O. ramosa* and 53.22% and 51.82% respectively for *O. crenata*). However higher concentrations of sulphur (1 and 2 g/L) significantly reduced the germination of the tested *Orobanche* seeds. No significant differences were detected between the germination percentages of any of the tested sulphur levels. Meanwhile these germination percentages were significantly less than those treated with GR_{24} treatments. Germination percentage of *Orobanche* seeds treated with water either alone or mixed wiuth any of the tested sulphur levels aer significantly less than these of seeds treated with GR_{24}. Germination percentage of *O. ramosa* significantly decreased by 4.00% and 1.75% in 0.25 g/L and 0.50 g/L respectively) and then increased by increasing the sulphyr concentration to 1.00 g/L (5.25%). The highest level of sulphur (2.00 g/L) gave comparatively considerable germination percentage (3.65%). Quite similar results were obtained in case of *O. crenata* (2.20%, 0.75%, 1.00%, 1.25% and 2.50% in case of seeds treated with water, 0.25, 0.50, 1.00 and 2.00 g/L of sulphur respectively).

According to the above mentioned results it could be concluded that radicle diffusates of turnip, fenugreek, coriander and lupins contain compound(s) which contradict the germination stimulatory effect of the synthetic compound GR_{24}. As a result, these crops could be used in intercropping system whith the susceptible economic host plants to reduce broomrape infection in their fields. Field experiments are needed ti detect the sowing date of the

intercropped plants and their seeding rates. It could be also concluded that the use of sulphur to control some pests other than broomrape (i. e. insects and fungi) in tomato fields may reduce the broomrape attachements on this economic crop. From the experimental point of view, coution must be taken in consideration when H_2O_2 and NaOCl used to sterilize *Orobanche* seeds.

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Table 1

GERMINATION PERCENTAGE OF *OROBANCHE* SEEDS AS AFFECTED BY TURNIP, FENUGREEK, CORIANDER AND LUPINS ROOT DIFFUSATES

Treatment	*O. ramosa*	*O. crenata*
GR_{24} 1ppm	80.16 (63.75) a	59.06 (50.37) a
TRD + GR_{24}	4.79 (42.50) c	38.13 (37.63) b
FRD + GR_{24}	56.75 (48.95) b	38.17 (38.12) b
CRD + GR_{24}	65.10 (53.80) b	34.86 (36.16) b
LRD + GR_{24}	57.77 (50.96) b	31.11 (33.87) b
L.S.D. 0.01	5.96	4.73
Water	9.47 (17.91) a	2.87 (9.75) a
TRD	2.75 (9.41) b	1.42 (6.54) b
FRD	2.22 (8.78) b	0.25 (6.12) b
CRD	5.00 (12.87) b	1.22 (6.03) b
LRD	5.15 (12.77) b	0.00 (6.25) b
L.S.D. 0.01	4.94	2.72

- Values between brackets are the arcsin square root of the germination percentage.

- Values followed by the same letter within the same column are not significantly different.

Table 2

GERMINATION PERCENTAGE OF *OROBANCHE* SEEDS AS AFFECTED BY HYDROGEN PEROXIDE, SODIUM HYPOCHLORITE AND SULPHUR

Treatment	Soaking time	*O. ramosa*	*O. crenata*
GR_{24} 1ppm		79.62 (63.25) a	60.42 (51.02) a
H_2O_2 10 V + GR_{24}	10	58.22 (49.26) b	23.00 (28.59) b
	20	40.25 (39.34) c	20.30 (26.73) c
	30	36.60 (37.22) c	19.62 (26.38) c
H_2O_2 15 V + GR_{24}	10	13.25 (21.16) d	0.00 (6.25) d
	20	1.75 (7.52) e	0.00 (6.25) d
	30	0.00 (6.25) e	0.00 (6.25) d
H_2O_2 30 V + GR_{24}	10	0.00 (6.25) e	0.00 (6.25) d
	20	0.00 (6.25) e	0.00 (6.25) d
	30	0.00 (6.25) e	0.00 (6.25) d
L.S.D.	0.01	2.56	1.47
GR_{24} 1ppm		80.07 (63.54) a	61.17 (51.46) a
NaOCl 1.30% + GR_{24}	5	66.07 (54.38) b	46.25 (42.82) b
	10	57.05 (49.08) c	30.87 (33.71) c
	15	41.95 (40.35) d	27.00 (31.38) c
NaOCl 2.70% + GR_{24}	5	14.17 (22.11) e	9.75 (17.93) d
	10	9.25 (17.65) f	0.00 (6.25) e
	15	3.50 (10.64) g	0.00 (6.25) e
NaOCl 5.25% + GR_{24}	5	0.00 (6.25) h	0.00 (6.25) e
	10	0.00 (6.25) h	0.00 (6.25) e
	15	0.00 (6.25) h	0.00 (6.25) e
L.S.D.	0.01	1.98	1.76
GR_{24} 1 ppm		79.87 (63.40) a	60.30 (50.94) a
GR_{24} + 0.25 S g/L		59.00 (50.18) b	53.22 (46.84) b
GR_{24} + 0.50 S g/L		57.40 (49.26) b	51.82 (46.02) b
GR_{24} + 1.00 S g/L		44.75 (41.97) c	43.75 (41.39) c
GR_{24} + 2.00 S g/L		45.55 (42.43) c	40.60 (39.54) c
Water		9.90 (18.33) d	2.20 (8.54) de
Water + 0.25 S g/L		4.00 (11.30) ef	0.75 (6.59) e
Water + 0.50 S g/L		1.75 (7.91) g	1.00 (7.05) de
Water + 1.00 S g/L		5.25 (13.28) e	1.25 (7.44) de
Water + 2.00 S g/L		3.65 (11.06) f	2.50 (8.84) d
L.S.D.	0.01	2.12	2.07

– Values between brackets are the arcsin square root of the germination percentage.
– Values followed by the same letter within the same column are not significantly different.

IV.4

GERMINATION OF *Orobanche crenata* SEEDS AT A WIDE RANGE OF ALTERNATING AND CONSTANT TEMPERATURES

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ABSTRACT

Germination of *Orobanche crenata* Forsk. was tested in darkness with GR24 at 169 different constant and alternating temperature regimes on a two-dimensional temperature gradient plate. Results were analysed in terms of characteristics of alternating temperature which appeared to control germination; i.e., mean temperature, amplitude, daily maximum temperature and thermoperiod (defined as the time spent at the maximum temperature each day). Germination was optimal at mean constant temperature of 18°C. Alternating temperatures appeared to be slightly promotive at very small amplitudes (defined as the difference between daily maximum and minimum temperatures) of about 2.5°C in 8h but not in 16h thermoperiod. Germination was, however, inhibited at higher amplitudes. In general, increase of maximum temperature (at a given mean temperature) decreased germination.

edge of the plate. The boxes (each containing 4 discs or about 100 seeds) were placed on to the moistened paper in each cell. Lids were placed on the boxes so that the seeds were kept moist at all times. The whole plate was covered with a single sheet of germination paper to prevent condensation. The paper was then covered with a sheet of polythene to minimise evaporation and another black polythene to prevent interference from light. The whole area was covered with a triple-glazed perspex (plexiglass) lid. The temperature gradient plate was kept in a dark and air-conditioned (25 °C) room.

Two experiments were conducted. Experiment 1 lasted 28 days and employed gradients of 7 - 40°C and experiment 2 lasted 29 days with gradients of 10 - 40 °C in both directions which were applied daily for 8 and 16 hours respectively. The seeds were therefore subjected to 13 constant and 156 alternating temperature regimes in two thermoperiods with variable maximum and minimum temperatures (Murdoch, *et al.*, 1989). Thermoperiod is defined as the time spent at the maximum temperature each day.

Results and Discussion

Maximum germination was found at a mean temperature of 18°C in experiment 1 (72.6%) (Fig. 1A) and 18.33°C in experiment 2 (73.8%) (Fig. 1B). The result was in agreement with Kasasian (1973) whose results show on average maximum germination at 18°C for seeds conditioned at temperatures between 8 and 28°C. More recently, Foy *et al.* (1991) reported maximum germination at 20°C and Van Hezewijk (1994) found high germination at 15-20°C in different seed lots of *O. crenata*.

Germination appeared to be controlled by maximum temperature and duration at which the seeds are exposed (thermoperiod). It can be observed from Figure 1 that at temperatures of more than 35°C for 16h thermoperiod, the seeds were totally inhibited from germinating even if the seeds were subjected to lower temperatures for the rest of the day. On the other hand, germination was higher at the lower thermoperiod. It can be concluded that the longer the seeds are exposed to higher temperature, the lower the germination.

The results of both experiments showed that at a given mean temperature, germination decreased with increase of amplitude (Fig 2). The only exception was with a 2.5°C amplitude at an eight hour thermoperiod where germination may have been slightly promoted by alternating temperatures at mean temperatures below 18°C (Fig 2A). However, at an amplitude more than 5°C, germination was inhibited. Van Hezewijk (1994), found that germination at 15/25°C (12h/12h) was approximately 58% compared to approximately 70% at 20°C constant implying that alternating temperatures were inhibitory with a 10°C amplitude which is consistent with our results. Although Foy *et al.* (1991) did not test germination at constant temperatures equivalent to the mean temperatures of alternating temperature regimes, it is nevertheless clear that a 10°C amplitude at 15/25°C or at 20/30°C (16h/8h) is inhibitory to germination of *Orobanche* seeds. By contrast, Racovitza (1959) reported that compared to 3°C, an 8°C temperature fluctuation favoured the germination and attachment of *O. ramosa* seeds. Racovitza used 21/24°C and 16/24°C regimes with mean temperatures of 22.5°C and 20°C, respectively (assuming 12h/12h cycles). His results are, therefore, consistent with ours since the lower mean temperature of the 16/24°C regime would be predicted to promote more germination (Fig 2).

An interaction of alternating temperatures and thermoperiod was observed at sub-optimal temperatures such that the 2.5°C amplitude, was promotive at an eight hour thermoperiod but inhibitory at the sixteen hour one.

In general, germination was highly influenced by the components of the alternating temperature regime. Germination was inhibited by temperature below and above the optimum (18°C) but the extent to which the inhibition occurred was modified by the duration of exposure to the

maximum temperature and the amplitude of the temperature fluctuation. Additional work on other species is currently underway which, after testing against the hypotheses outlined, could be used in predicting patterns of germination in the field.

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IV.5

GERMINATION OF *Striga hermonthica* SEED AT CONSTANT TEMPERATURES

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ABSTRACT

The effect of a range of constant temperatures (15-45^{0}C) on the germination of a *S. hermonthica* (Del.) Benth. seed lot was investigated on a temperature gradient plate. The seeds were conditioned in water at 20^{0}C for 15 d and a synthetic stimulant, GR-7, added before applying the temperature treatments. Positive linear relationships were established between the rate (reciprocal of time taken) of germination of 85, 90 and 95% of the final percentage germina- tion and temperature. Germination occurred at temperatures between 25^{0}C and 42.5^{0}C, but the rate was fastest at 40^{0}C. The estimated base temperature, T_b, at which germination was zero was about 22.1-22.7^{0}C for the three percentages enumerated above. Germination rate was also optimal at 40^{0}C, T_o, but declined thereafter showing that the ceiling temperature, T_c, at which germination was again zero, was >42.5^{0}C. Lag time varied from about 10 h for the temperature range of 30 to 42.5^{0}C to about 48 h for the 25 to 27.5^{0}C temperatures. The final percentage germination was highest at 40^{0}C and lowest at 25^{0}C. The cardinal temperatures for germinating *S. hermonthica* from this study were: T_b = 22.1-22.7^{0}C, T_o = 40^{0}C and T_c >42.5^{0}C compared with T_b of 18.9-20.8, T_o 32-35 and T_c >42.5^{0}C for seeds conditioned at 30^{0}C in an earlier study.

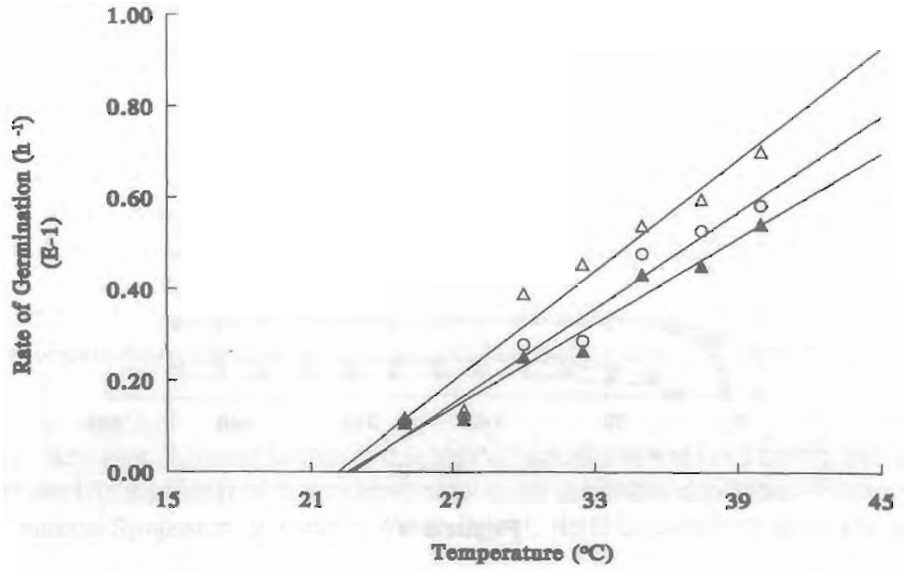

Figure 3

Effect of temperature on the rate of germination of 85% [Gt85 (△)], 90% [Gt90 (○)] and 95% [Gt95 (▲)] of the final germination percentage of *S. hermonthica* seed. The fitted lines give R^2 = 0.9505, 0.9565 and 0.9594; base temperatures of 22.1, 22.7 and 22.5 and thermal times, θ_T, 248, 287 and 323.6°Cd, respectively for Gt85, Gt90 and Gt95.

IV.6

DIFFERENCES IN THE PRIMARY DORMANCY PATTERN OF *Striga* SPECIES; AN ONGOING STUDY

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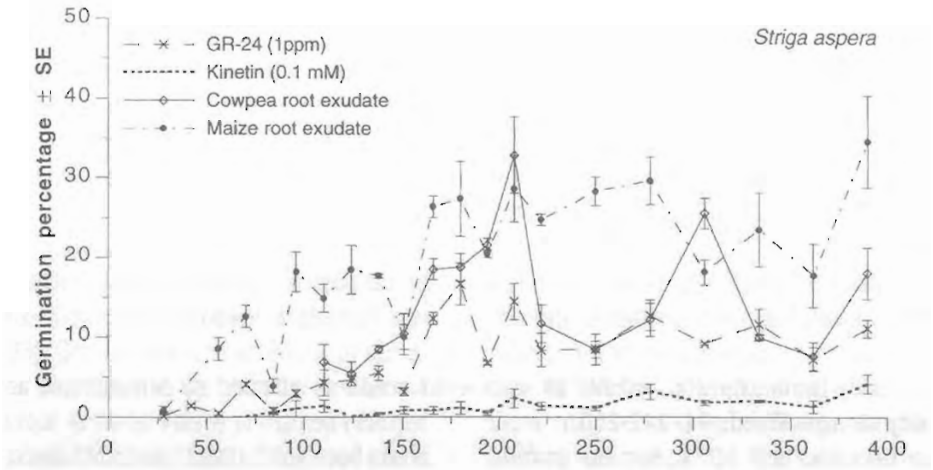
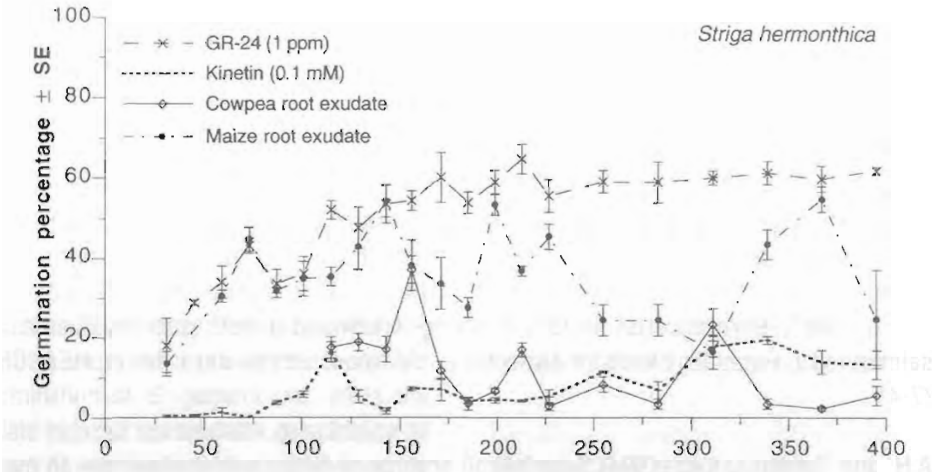
ABSTRACT

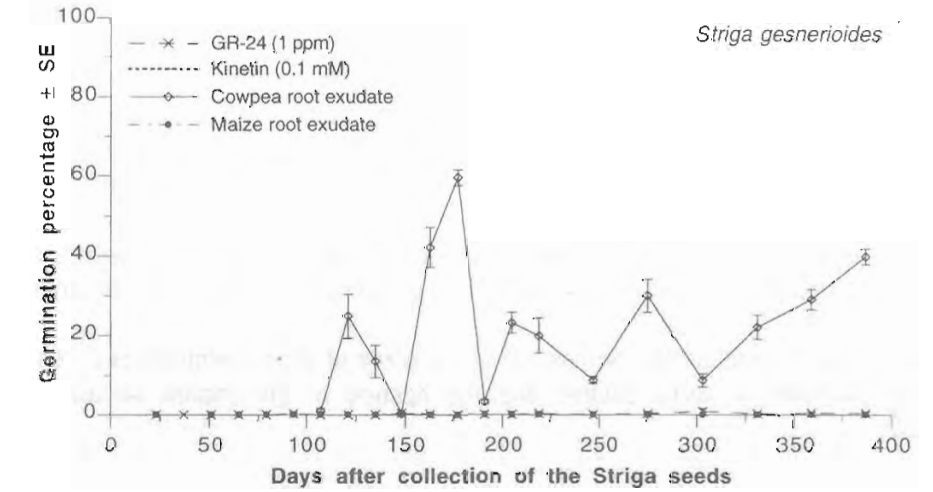
Primary dormancy was tested in populations of *Striga asiatica*, *S. aspera*, *S. brachycalyx*, *S. gesnerioides* and *S. hermonthica*, collected in West and East Africa. Four different germination stimulants were used: GR-24, kinetin, cowpea root exudate and maize root exudate. In all the *Striga* species tested primary dormancy was observed. However, there were marked differences in the length of the primary dormancy period and in the response to the germination stimulants. Dormancy of *S. aspera* and *S. hermonthica* seeds was not absolute and lasted less than four months. On the other hand, seeds of *S. gesnerioides* and *S. brachycalyx* underwent absolute dormancy during approximately four months, and part of the seeds remained dormant during six to seven months. Within *S. asiatica*, population specific differences occurred. In a population from Mali there was absolute dormancy during a one year period, whereas the length of the primary dormancy period in populations from Kenya and Tanzania was much shorter.

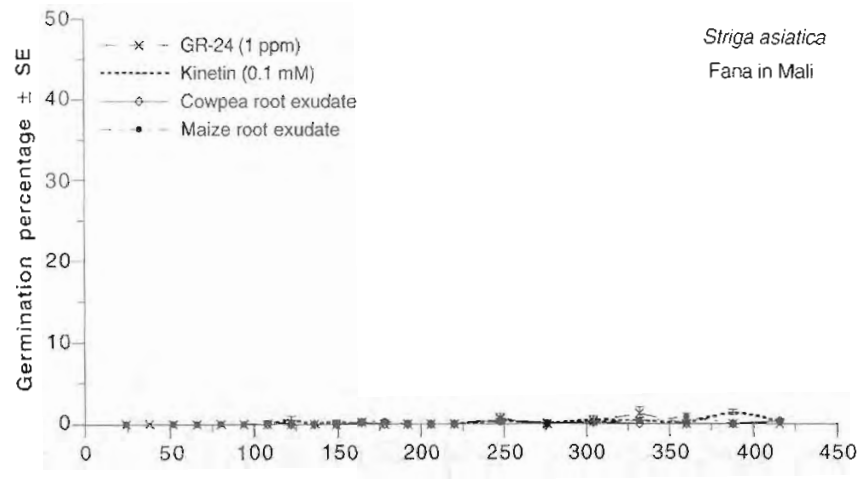
The differences in primary dormancy pattern could not be related to the rainfall in the areas were the seeds were collected. However, there are indications that seed size could play a role, especially within *S. asiatica*.

Additional Key words: germination, host specificity, root exudates, variability.

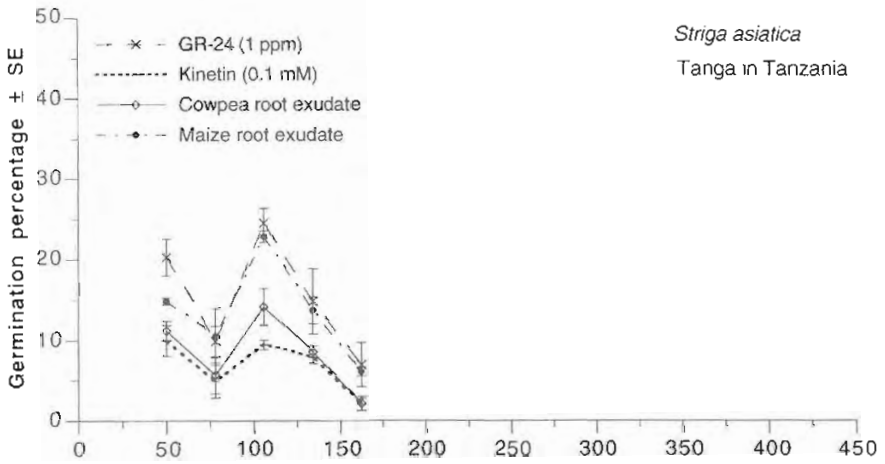

Striga aspera
GR-24 (1ppm)
Kinetin (0.1 mM)
Cowpea root exudate
Maize root exudate
Germination percentage ± SE
0
10
20
30
40
50
0
50
100
150
200
250
300
350
400


Striga hermonthica
GR-24 (1 ppm)
Kinetin (0.1 mM)
Cowpea root exudate
Maize root exudate
Germination percentage ± SE
0
20
40
60
80
100
0
50
100
150
200
250
300
350
400

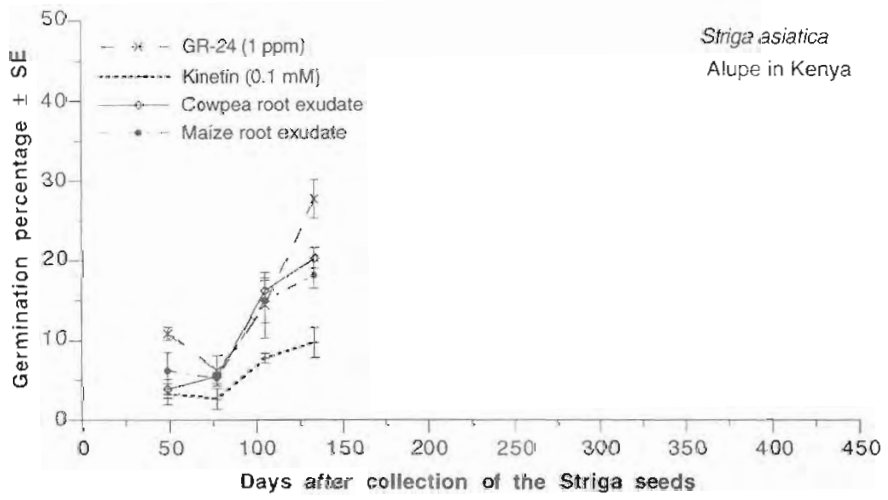

Striga gesnerioides
GR-24 (1 ppm)
Kinetin (0.1 mM)
Cowpea root exudate
Maize root exudate
Germination percentage ± SE
0
20
40
60
80
100
0
50
100
150
200
250
300
350
400
Days after collection of the Striga seeds



Striga asiatica
Fana in Mali



Striga asiatica
Tanga in Tanzania



Striga asiatica
Alupe in Kenya

Introduction

In a recent overview of the germination ecology of *Striga* and *Orobanche*, data were presented on the occurrence of primary dormancy in *Striga hermonthica* (Del.) Benth. seeds (Pieterse and Verkleij 1994). In most cases these seeds undergo a condition of primary dormancy, however, it was concluded that no generalizations should be made. It seems likely that primary dormancy in *S. hermonthica* will vary, due to an adaptation to different environmental conditions. The present study is concerned with the occurrence of primary dormancy in *S. hermonthica* seeds in the two northern provinces, Atacora and Borgou, of the Republic of Benin. Seeds were collected at eight different sites, from three different crops, maize, pearl millet and sorghum. Research was conducted in the framework of a broader study on seed viability and germination of *S. hermonthica*, sponsored by the European Union. The ultimate objective is to come to a more effective application of cultural control methods, such as rotation with trap crops.

Materials and Methods

Seeds of *Striga hermonthica* (Del.) Benth. were collected at different sites from maize, pearl millet and sorghum in the Atacora and Borgou provinces in November 1994. See for the provenance of these seeds Table 1. In these provinces the rainfall pattern is monomodal.

The seeds from the different sites were kept under dry conditions at room temperature in the laboratory ("laboratory seeds") at the Ina Research Station, situated 70 km north of Parakou in Borgou Province. In addition, part of the seeds collected in the field at Ina Station were put in 4 x 9 cm nylon gauze bags (approximately 1,000 seeds per bag). These bags were subsequently buried in the field at Ina Station. There were two replicates of 25 bags for seeds collected from *S. hermonthica* plants parasitizing maize ("*Striga*/maize strain"), as well as for seeds collected from *S. hermonthica* plants parasitizing sorghum ("*Striga*/sorghum strain"). Each bag was buried at a depth of 5 cm in the middle of a 0.5 x 0.5 m plot. The plot was kept weed-free.

Germination rates of "laboratory seeds", as well as those of "field seeds", were regularly tested. For each test the seeds were first conditioned (exposure to moist conditions): approximately a thousand "laboratory seeds" per sample and "field seeds" from four exhumed bags of the "*Striga*/maize strain" and from four bags of the "*Striga*/sorghum strain". "Field seeds" were also tested for viability by means of the tetrazolium colour test.

Before conditioning the seeds were spread on a filter paper and surface sterilized for 5 minutes with a 1% solution of commercial sodium hypochlorite (NaOCl). Subsequently, the seeds were thoroughly rinsed with distilled water and for conditioning transferred to a 47 mm diameter glass fibre filter paper moistened with 1 ml of 0.3 mM MES [2(N-morpholino) ethane sulphonic acid] buffer in a petri dish (60 mm diameter). The petri dish was sealed with parafilm, wrapped in aluminium foil and subsequently kept in an incubator at $30^0 \pm 5^0$C for 14 days.

After conditioning, approximately three hundred seeds of each "laboratory seed" sample were placed on a 47 mm glass fibre filter paper disk in a petri dish (60 mm diameter) for the germination test. Each sample was divided into three clusters of approximately a hundred seeds, which were considered to be replicates. To trigger germination, 1 ml of a 1 ppm solution of GR24 (a synthetic germination stimulant) was added to the petri dish. (For the preparation of the 1 ppm GR24 solution see Gbèhounou *et al.* 1996). The petri dish was wrapped in aluminium foil and incubated for 48 hours at $30^0 \pm 5^0$C. At the end of the incubation period, the petri dish was opened, and the germinated seeds were counted under a microscope. The germination test of "field seeds" was performed in the same way. Approximately a hundred seeds of each bag was tested in a petri

dish. To assess the viability of "field seeds", 1.5 ml of a 1% tetrazolium salt solution (for preparation see Gbèhounou *et al.* 1996) was added to approximately one hundred conditioned seeds (spread on 47 mm diameter glass filter paper in a 60 mm diameter petri dish). These petri dishes (sealed with parafilm and wrapped in aluminium foil) were opened after 8 days' incubation at $30^{0}\pm5^{0}$C, and subsequently coloured (pink or red) and non-coloured seeds were counted under a microscope. The coloured seeds were assumed to be viable.

Results

The germination rates of seeds collected in the field, which were directly tested in the laboratory ("laboratory seeds") are shown in Fig. 1 (seeds collected from maize and pearl millet) and Fig. 2 (seeds collected from sorghum).

On the first testing date (February 10), the percentages of germinating seeds of *Striga* plants collected from maize was 25% (Bagou), 20% (Guéné) and 4% (Ina), respectively (see Fig. 1). Subsequently, these percentages tended to decrease and in April they were 2%, 2% and 0.5%, respectively. However, at the end of May the percentages had increased to 80%, 54% and 60%, respectively. These high percentages were also found in the beginning of July and in August.

Seeds collected from *Striga* plants parasitizing pearl millet did not germinate (with the exception of a few seeds) until May (see Fig. 1). The percentages at the end of May were 71% (Guéné) and 35% (Kobly), respectively. At the beginning of July the germination percentages had markedly decreased.

Percentages of germinating seeds of *Striga* plants collected from sorghum were relatively low on the first testing date (February 10) (see Fig. 2ab). The highest value was 15% (Tanguiéta) and the lowest value 0% (Kobly). On subsequent testing dates these values remained low until in April a low was reached of 0% in most collections. There were only a few germinating seeds in April in the collections from Bagou (0.1%), Kandi (0.5%) and Kobly (3.5%). At the end of May the germination rate of the seeds from all collections was relatively high. The highest percentage in May was 77% (Ina) and the lowest 57% (Kandi). Similar percentages of germinating seeds were obtained at the beginning of July and in August.

Germination and viability rates of seeds kept in nylon gauze bags in the field at Ina Station are shown in Fig. 3. On the first testing date (December 12), the percentages of germinating seeds were 35% for *Striga* seeds collected from maize and 27% for *Striga* seeds collected from sorghum. The percentages of viable seeds were 44% and 41%, respectively. Subsequently, germination values decreased and in April they were as low as 4% for *Striga* seeds from maize and 2% for *Striga* seeds from sorghum. On the other hand, the viability values showed an increase and in April they were 71% for *Striga* seeds from maize and 89% for *Striga* seeds from sorghum. At the end of May there was an abrupt increase in the germination rate, 53% of the *Striga* seeds from maize and 55% of the *Striga* seeds from sorghum had germinated. After April the viability values decreased markedly and in June the percentages were 22% for *Striga* seeds from maize and 26% for *Striga* seeds from sorghum.

Discussion

Results show that during the first five months after shedding, which approximately covers the period November - April, *S. hermonthica* seeds in Benin pass through a process of primary dormancy. In May, which coincides with the beginning of the cropping season, a sharp increase in germination rates was observed. In contrast to *Striga* seeds from pearl millet, primary dormancy was not absolute for *Striga* seeds collected from maize, and to a lesser extent, not for *Striga* seeds from sorghum. It seems that these differences are not correlated with the location's latitude. However, it is conceivable that genetic differences between the

Striga populations play a role in this respect, which could be connected with adaptations to the different host crops.

It is remarkable that before primary dormancy was terminated in May, germination rates in those populations where dormancy was not absolute, tended to decrease until a low was reached in April. The cropping season in Northern Benin usually starts at the end of May or beginning of June. Earlier sowing is too risky for farmers, due to the irregular rain pattern. However, conditioning of *Striga* seeds in the soil will start after the first rains which, depending on the latitude, may already fall in March or April (Gbèhounou *et al.* 1994). Consequently, it could be hypothesized that the survival strategy of *S. hermonthica* seeds is particularly aimed at preventing germination early in the rainy season.

Seeds kept in nylon bags in the field at Ina Station ("field seeds") showed a similar primary dormancy pattern as seeds kept in the laboratory ("laboratory seeds"). In general, "viability" (tetrazolium colouring) of these seeds seemed to increase during the dormancy period. However, it should be noted that there was a large variability and that the TTC test is not quite reliable. Decreased viability of non-dormant seeds, which became apparent in May and June, could be connected with the dying off process of *S. hermonthica* seeds in the course of the rainy season (Gbèhounou *et al.* 1994; Gbèhounou *et al.* 1996).

Acknowledgment

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IV.8

GERMINATION AND VIABILITY OF *Striga hermonthica* SEEDS IN WESTERN KENYA IN THE COURSE OF THE LONG RAINY SEASON

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ABSTRACT

Field studies were conducted in Western Kenya to investigate the germination and viability rate of *Striga hermonthica* (Del.) Benth. seeds in the course of the long rainy season of 1995. At the beginning of this season seeds were put in small nylon bags which were buried in a field which was kept weed-free. Subsequently, the bags were regularly dug up and germination and viability of the seeds was tested in the laboratory. Initial germination rate was relatively high. However, after one to three weeks storage in the field the number of germinating seeds decreased steadily, and after 12 weeks, germination approached 0%. Viability also decreased and after 12 weeks most of the seeds had died. It appeared that many seeds had already germinated in the field, in spite of the apparent absence of host plants, and that most dead seeds were in fact empty. Five weeks after the start of this experiment, *S. hermonthica* seeds were also buried in a field treated with methyl bromide. For a period of nine to twelve weeks there was no marked decrease in the number of germinating seeds in this field and the number of empty seeds remained relatively low. Although the two experiments were not conducted simultaneously, it seems likely that the number of dead seeds in non-fumigated soil is connected with an effect of soil-organisms on *Striga* seeds.

Additional key words: parasitic weed, seed ecology, cereals, East Africa.

Introduction

Recently it was observed that germination and viability of *Striga hermonthica* (Del.) Benth. seeds in the Republic of Benin decreased steadily in the course of the rainy season (Gbèhounou *et al.* 1994; Gbèhounou *et al.* 1996). This was due to a dying off process and the seeds did not enter a stage of secondary dormancy. These results do not seem to be in accordance with earlier work of Vallance (1950), who reported that after prolonged conditioning under laboratory conditions *S. hermonthica* seeds become dormant. This secondary dormancy, which Vallance (1950) called "wet dormancy", could be broken by drying the seeds and repeated conditioning. Although decreased germination in vitro as a result of prolonged conditioning was confirmed by subsequent researchers, it was not clarified whether this was due to secondary dormancy or to dying off of the seeds (see Pieterse and Verkleij 1994).

To determine whether seed mortality in the field during the rainy season is a phenomenon which also occurs in other regions, germination rate and viability of *S. hermonthica* seeds in the western part of Kenya were assessed during the long rainy season of 1995.

Materials and Methods

Striga hermonthica seeds were used which were collected at the end of the long 1994 rainy season in July 1994 and at the end of the 1994 short rainy season in January 1995.

Trials were conducted at Kibos, the KARI National Sugar Research Centre near Kisumu in Nyanza Province (latitude $0^{o}4$'S; longitude $34^{o}48$'E; altitude 1214 m). In the first trial, which started on March 14, 2 x 2 cm nylon gauze bags containing *S. hermonthica* seeds were buried in a 9 x 3 m field. In previous years sorghum had been grown at this experimental site. However, during the 1994 short rainy season the field had been left fallow. As it was overgrown by wild plants the field was cleared by means of hoeing and subsequent ploughing. Plant roots were removed as much as possible before burying the bags and during the experiment the field was kept weed-free. The field was divided into three 9 m^2 plots and, to separate these plots, only 4 m^2 of each plot was used.

Seeds of each cohort were put in 54 nylon gauze bags (approximately 1,500 seeds per bag). Subsequently, in each plot 36 bags (18 bags of each cohort) were buried at a depth of 10 cm. These bags were connected by a string to marking sticks put into the soil (each stick was connected to two bags, one containing "long rainy season" seeds and one containing "short rainy season" seeds). Once a week two bags connected to the same stick were dug up from each plot. The bags were transferred to the laboratory where they were opened and the seeds removed. Subsequently, seeds from each bag were washed and divided into three groups. The seed germination rate of seeds of the first group was immediately tested in the laboratory with the synthetic germination stimulant GR-24 (Gbèhounou *et al.* 1996). In addition to counting the number of germinating seeds, after May 15 also the number of empty seed coats was determined. The second group of seeds was conditioned after surface sterilization (Gbèhounou *et al.* 1996). Subsequently, germination of one half of the seed sample was assessed by means of GR-24, whereas the other was tested by means of ethylene gas. The ethylene treated petri dishes were kept in a separate room. The third group of seeds was used for a tetrazolium colour test (see Gbèhounou *et al.* 1996).

In the second trial, which started on April 18, a 6 x 2 m weeded field was used. On March 15 this plot had been treated with 86 g methyl bromide gas per m^2. The field was divided into three 2m^2 plots and for the experiment 1.5m^2 in each plot was used. Nylon gauze bags with seeds from the two cohorts were buried in the same way as described for the first trial. The number of germinated seeds was assessed, after conditioning, by means of ethylene injection. In addition, empty seed coats were counted.

Results

Results of the direct germination tests are shown in Fig. 1 ("short season seeds") and Fig. 2 ("long season seeds"). During the first weeks the percentage of germinating seeds increased markedly, which may be attributed to conditioning. For the "short season seeds" a maximum of 50% was reached after 4 and 5 weeks, for the "long season seeds" a maximum of 27% was reached after 5 weeks. Subsequently, the percentage of germinating seeds decreased steadily, until at the end of the rainy season germination became 0%. In the course of the experiment the number of empty seed coats increased and at the end of the rainy period all seeds were empty.

Results of germination tests with conditioned seeds are presented in Figs. 3 and 4. These results are similar to those of the direct germination test. However, the first measurement (directly after conditioning) already revealed a relatively high germination rate. If exposed to GR24 this was 50% for "short season seeds" and 64% for "long season seeds". When germination was triggered with ethylene, these percentages were 78% and 68%, respectively. However, after the seeds had been stored for some time in the field, the number of germinated seeds triggered by ethylene was not markedly higher than the number triggered by GR24. The decrease in germination of "short season seeds" became apparent after approximately three weeks, whereas germination of "long season seeds" already decreased markedly after one week. According to the TTC test seeds died in the course of the rainy season. Whether this was due to suicidal germination or to another effect was not tested. However, the seeds were taken from the same samples which were used for the direct germination tests. As a large number of these seeds was empty (Figs. 1 and 2) and various germinating seeds were observed after opening the nylon bags, it may be assumed that many dead seeds (not coloured by the TTC test) were in fact empty.

The germination rate of seeds kept in bags in fumigated soil did not show a decline during the first 9 weeks (for "short season seeds") and the first 12 weeks (for "long season seeds"), respectively. Very few empty seeds were found in these samples.

Discussion

In accordance with previous findings in the Republic of Benin (Gbèhounou *et al.* 1996), it may be concluded that under field conditions in Kenya *S. hermonthica* seeds die in the course of the rainy season. However, the dying off process seems to be more rapid in Kenya than in Benin. Most seeds had died after 10 to 14 weeks, whereas in Benin the percentage decreased from 82% to 16% in the course of 23 weeks. These observations seem to be substantiated by results of a recent long-term field study at the same site in Kenya, which also point to a rapid decline in the *Striga* seed bank (Odhiambo and Ransom 1995).

There are strong indications that a large number of seeds in Kenya dies as a result of suicidal germination. Many dead seeds appeared to be empty and a relatively large number of germinating seeds was observed in the nylon bags. In Benin it was not studied whether or not dead seeds were empty.

As the seeds did not seem to die in plots treated with methyl bromide, at least not within 9 to 12 weeks, it could be that decreased seed viability in the field is connected with the activity of living organisms in the soil. It is conceivable that micro-organisms in the soil produce ethylene or that remnants of plant roots produce natural germination stimulants. However, it should not be ruled out that seeds may also die as a result of direct infestation by micro-organisms. As the experiment on fumigated soil started five weeks after the experiment on non-fumigated soil an effect of environmental factors other than soil organisms cannot entirely be excluded. However, climatological conditions during the long rainy season do not vary substantially. Methyl bromide is used to control fungi, nematodes and weeds

(Worthing and Hance 1991). It is not effective against bacteria.

It may be concluded that the germination pattern of "short season seeds" was similar to that of "long season seeds". Therefore it seems likely that under conditions prevailing in Western Kenya no adaptation of *S. hermonthica* strains has taken place to the short rainy season or to the long rainy season. This is in agreement with earlier work of Ransom and Njoroge (1991), who found that the after-ripening requirement of *S. hermonthica* seeds does not preclude seeds produced in one season from germinating in the following season. It is obvious that the low germination rate of seeds which had briefly been kept in the field was connected with insufficient conditioning. If these seeds were conditioned in the laboratory, their germination rate was relatively high.

At the beginning of the rainy season ethylene proved a more effective germination stimulant than GR24. However, this difference was not apparent after seeds had been stored in the field for some weeks. This is in accordance with a previously, unpublished observation made by A.H. Pieterse, P. Scheppers, J.K. Ransom, G. Odhiambo, S.J. ter Borg and J.A.C. Verkleij (1992). As this phenomenon was observed for "long season seeds" as well as for "short season seeds", already collected in July of the previous year, it seems that this is not connected with a more effective breaking of primary dormancy by ethylene.

Results do not indicate that seeds enter a state of secondary dormancy. As the seeds rapidly die in the course of the rainy season, crop rotation could be an effective control method under field conditions in Western Kenya.

Acknowledgment

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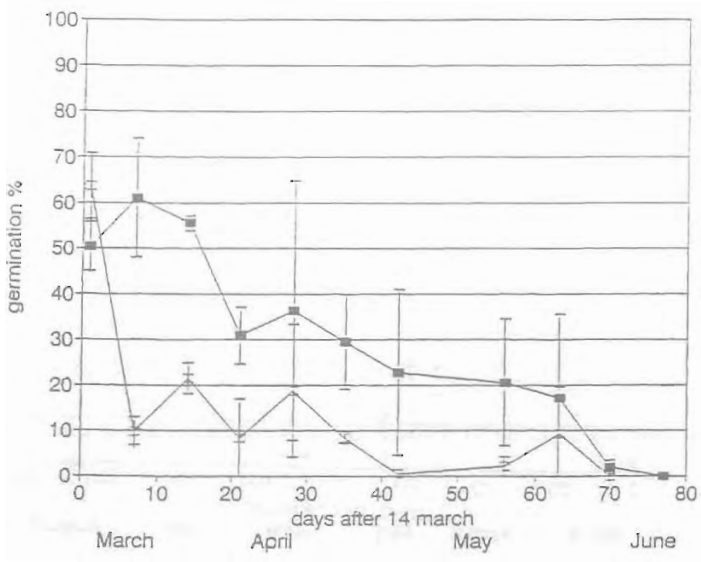

Figure 3

Germination rates of *Striga hermonthica* seeds, collected at the end of the short rainy season, (indicated by black squares) and at the end of the long rainy season (indicated by empty squares), after conditioning in the laboratory. Tests were conducted with GR-24 in the period March - August 1995. Vertical bars indicate standard deviation above and below the mean.

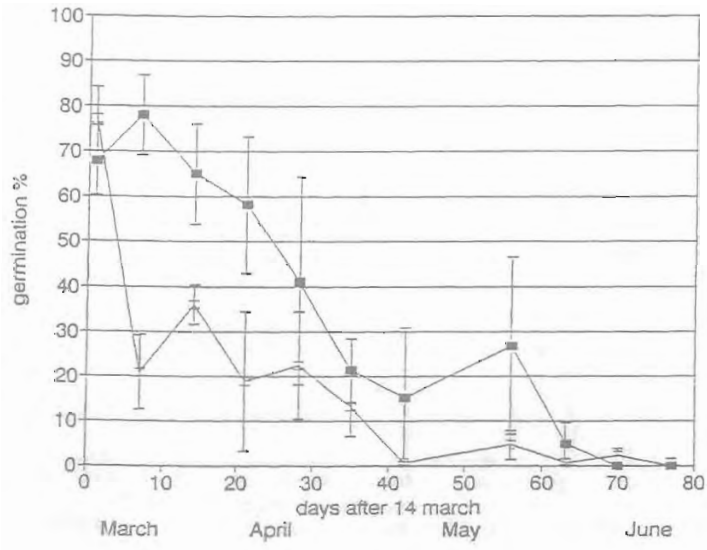

Figure 4

Germination rates of *Striga hermonthica* seeds, collected at the end of the short rainy season, (indicated by empty squares) and at the end of the long rainy season (indicated by empty squares), after conditioning in the laboratory. Tests were conducted with ethylene in the period March - August 1995. Vertical bars indicate standard deviation above and below the mean.

IV.9

HOSTS OF EIGHT *Striga* SPECIES (SCROPHULARIACEAE) IN CAMEROON

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ABSTRACT

Host ranges of *Striga aspera* (Willd.) Benth., *S. brachycalyx* Skan. *S. forbesii* Benth., *S. gesnerioides* (Willd.) Vatke, *S. hermonthica* (Del.) Benth., *S. ledermannii* Pilger, *S. macrantha* Benth., and *S. passargei* Engler were studied in northern Cameroon. As expected, most hosts were grasses except for *S. gesnerioides.* Caryophyllaceae and Apiaceae are documented as the first dicot host families for *S. aspera.* Two ecotypes of *S. aspera* are noted.

Additional key words: witchweeds, host selection/range.

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Table 1

DOCUMENTED HOSTS OF *STRIGA* SPECIES IN NORTHERN CAMEROON

Striga aspera	*Alysicarpus ovalifolius* (S. et Th.) Leon
	Andropogon fastigianus Sw.
	A. laxatus Stapf
	Aristida hordeacea Kunth
	A. kerstingii Pilger
	Brachiaria comata (A.Rich.) Stapf
	B. villosa (Lam.) A.Camus
	Bulbostylis coleotricha (A.Rich.) C.C.Cl.
	Commelina benghalensis L.
	Dactyloctenium aegyptium (L.) P.Beauv.
	Digitaria sp.
	Echinochloa colona (L.) Link
	Eragrostis tremula Hochst. ex Steud.
	Fimbristylis cioniana Savi
	F. hispidula (Vahl) Kunth
	Hackelochloa granularis (L.) O.Ktze
	Loudetia togoensis (Pilger) C.E.Hubbard
	Polycarpaea corymbosa (L.) Lam.
	Schizachyrium exile (Hochst.) Pilger
	S. nodulosum (Hack.) Stapf
	Setaria anceps Stapf ex Massey
	Spermacoce radiata (DC.) Sieber ex Hiern
	S. microprotus
	Zea mays L.
Striga brachycalyx	*Schizachyrium sanguineum* (Retz.) Alston
	Loudetia togoensis
Striga forbesii	*Brachiaria brizantha* (Hochst. ex A.Rich.) Stapf
	Echinochloa colona
	E. obtusiflora Stapf
	Fimbristylis hispidula
	Setaria anceps
Striga gesnerioides	*Centella asiatica* (L.) Urb.
	Desmodium hirtum Guill. & Perr.
	Indigofera hirsuta L.
	I. prieureana Guill. & Perr.
	I. secundiflora Poir.
	I. stenophylla Guill. & Perr.
	Indigofera sp.
	Ipomoea coscinosperma Hochst. ex Choisy
	Tephrosia linearis (Willd.) Pers.
	T. pedicellata (Bak.) C.E.Hubbard

Introduction

Host preference or physiological specialisation in the genus *Striga* has been reported several times. The first observation of the existence of "biological strains" of *Striga asiatica* was in the early thirties by Lewin (Jones, 1955). Jones reported the presence of two host-specific *S. hermonthica* forms in the Sudan; one was preferably attacking sorghum, and the other *Pennisetum.* The existence of at least two distinct "races" of *S. hermonthica* in West Africa, one parasitizing pearl millet and the other sorghum, was described by King and Zummo (1977). They also identified a possible third race, primarily parasitic on maize. Host specificity depending on a germination stimulant was demonstrated by Parker and Reid (cf. Vasudeva Rao and Musselman, 1987).

Preliminary evidence for cultivar-specific physiological variants of *S. hermonthica* was described with respect to germination. Samples of 11 *S. hermonthica* populations from sorghum and pearl millet were allowed to germinate in the presence of root exudates of 27 sorghum populations (Bebawi, 1981). This experiment showed that sorghum cultivars differ in their ability to stimulate *Striga* seed germination, providing additional evidence for the existence of intraspecific variants of *S. hermonthica.* Both intercrop and intracrop adaptation of *S. hermonthica* were observed in three cereal crops (maize, sorghum, and millet) in an experiment in Nigeria (Kim, 1994). Adaptation of *S. hermonthica* to specific host crops (three pearl millet and one sorghum cultivar) was also reported in Niger (Hess, 1994). The study presented here was a continuation of this factorial infestation experiment. We investigated the parasitic abilities of *S. hermonthica* collected from millet and sorghum at different locations in Niger. Through a factorial pot experiment we wanted to determine to what extent *Striga* populations harvested from millet and sorghum were adapted to their corresponding host.

Materials and Methods

Seeds of seven *Striga hermonthica* populations were used in this study. They had been collected in 1991 from farmers fields in five locations in Niger: Bengou, Guéza, Sadoré, Birni N'Konni and Maradi (Figure 1). In all five locations pearl millet is the most important cereal species. At four locations (Bengou, Guéza, Birni N'Konni and Maradi) farmers additionally cultivate sorghum. In two of these four locations (Bengou, Birni N'Konni) *Striga* attacks sorghum. *Striga* populations were subdivided in two main groups, millet-*Striga* and sorghum-*Striga.* Host crops used in the experiment were exclusively landraces collected in the same locations as the *Striga* populations (Table 1). Studies were done at two levels of infestation: level one with 1,000 viable seeds per pot and level two with 16,000 viable seeds per pot. Nearly uniform infestation was obtained by estimating the viability of seeds and counting the number of *Striga* seeds per mg. The triphenyl-tetrazolium-chloride test (TTC-test) was used to estimate the viability (Linke, 1987).

The trial was conducted outdoors in pots at ICRISAT (International Crops Research Institute for the Semi-Arid Tropics) Sahelian Centre in Sadoré (near Niamey, Niger) during the period of May to December in 1994. Eleven-liter-pots were used filled with a *Striga*-free soil mixture (1/4 farmyard manure, 5/8 river sand and 1/8 clay). Developmental and virulence characters were studied during the experiment in weekly intervals. Traits studied in *Striga* were date of emergence, date of flowering, number of emerged and flowering plants per pot, and dry biomass at harvest. In addition the date of heading, plant height, dry matter weight, and grain yield of host plants were recorded. The experimental design was a completely randomised block design with four replications. All 126 factor combinations (seven local *Striga* populations x nine hosts x two infestation levels) were used and additionally one uninfested check per host population per replication. A total of 540 pots were used. To infest the pots the upper 1 cm

ACKNOWLEDGMENTS

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Table 2

IN VITRO PATHOGENICITY TEST OF SOME SOILBORNE FUNGI ON *OROBANCHE* SPP.

Map Location	Governorate and Host	Infection degree on *Orobanche* tissue		
		High	Moderate	Weak
Northern Egypt	1-Beheira	*Trichoderma*	*Fusarium*	*Aspergillus*
	Pea	*Fusarium*	*Penicillum*	
		Alternaria		
	2-Dakahlia			*Aspergillus*
	Faba bean	*Trichoderma*	*Fusarium*	*Fusarium*
				Trichoderma
	3-Gharbia			
	Cabbage	–	–	*Aspergillus*
				Fusarium
	Cauliflower	*Fusarium*	–	*Penicillum*
	Tomato	*Trichoderma*	*Trichoderma*	
Middle Egypt	4-Bani-seuf			
	Camomile	*Fusarium*	–	–
	Geranium	–	*Fusarium*	*Penicillum*
	Faba bean	*Alternaria*	*Fusarium*	–
Souther Egypt	5-Assiut			
	Faba bean	*Trichoderma*	*Trichoderma*	*Aspergillus*
			Fusarium	

Table 1

FUNGAL SCREENING IN FIVE EGYPTIAN GOVERNORATES IN CONNECTION TO MAP LOCATION, BROOMRAPE INFECTION AND HOST

Map Location	Governorate Rhizosphere samples location	Most frequent	Host under test
Northern Egypt	1-Beheira		
	infected (A)	*Fusarium; Aspergillus*	Pea
	uninfected (B)	*Penicillum*	Pea
	2.-Gharbia		
	infected (A)	*Fusarium; Aspergillus*	Cabbage, Cauliflower, Tomato
	uninfected (B)	*Trichoderma*	Cabbage, Cauliflower, Tomato
Middle Egypt	3-Dakahlia		
	infected (A)	*Fusarium; Aspergillus*	Faba bean
	uninfected (B)	*Penicillum and Cladosporium*	Faba bean
Souther Egypt	4-Bani-Seuf		
	infected (A)	*Fusarium; Aspergillus*	Camomile, Geranium, Faba bean
		Alternaria	Faba bean
	uninfected (B)	*Penicillum and Trichoderma*	Camomile, Geranium
	5-Assiut		
	infected (A)	*Fusarium; Aspergillus*	Faba bean
	uninfected (B)	*Penicillum and Rhizopus*	Faba bean

Table 1

MAXIMUM VALUES OF *STRIGA HERMONTHICA* INFECTION AND INFESTATION BY *SMICRONYX* ADULTS, GALLS AND *JUNONIA ORITHYA* LARVAE IN 8 FARMERS'FIELDS IN NORTHERN GHANA,1994

Field-No.	Striga/qm	Striga plants infested with Smicronyx adults (%)	Strigaplants infested with Smicronyx galls (%)	Galls per Striga plant (%)*	Striga plants infected with Junonia larvae (%)
1	7.6	14	21	78.0	14
2	6.0	15	29	100.0	10
3	2.7	12	35	71.7	5
4	2.0	19	19	83.7	12
5	0.5	22	5	41.2	15
6	0.6	2	2	100.0	6
7	1.3	23	41	66.9	8
8	1.2	21	28	76.5	20
MEAN	2.7	16	22.5	77.3	11.3

*) *calculated as the percentage of the total number of seed capsules.*

Table 2

IMPACT OF *SMICRONYX* SPP. ON SEED PRODUCTION OF *STRIGA HERMONTHICA*

Number of *Striga* plants per m^2	2.7
Striga plants per ha	27,000
Viable seeds per *Striga* plant (Sprich. 1994)	23,119
Viable seeds per ha without *Smicronyx* infestation	624,213,000
Striga plants infested with *Smicronyx* galls (%)	22.5
Healthy *Striga* plants per ha	20,925
Viable seeds produced by healthy plants	483,765,075
Number of infested *Striga* plants per ha	6,075
Smicronyx galls per *Striga* plant (%)	77.3
Viable seeds per infested *Striga* plant	5,257
Viable seeds produced by infected *Striga* plants	31,937,858
Viable seeds per ha with *Smicronyx* infestation	515,702,933
Reduction of *Striga* seed production by *Smicronyx* (%)	17.4

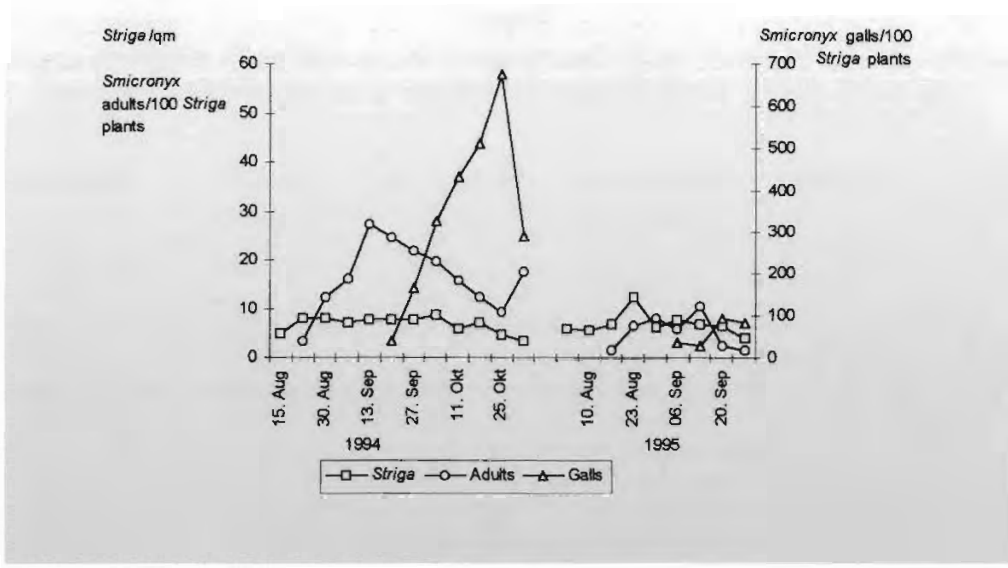

Figure 1

Population dynamics of *Smicronyx* spp. in northern Ghana in 1994 and 1995 in eight and two farmers' fields, respectively.

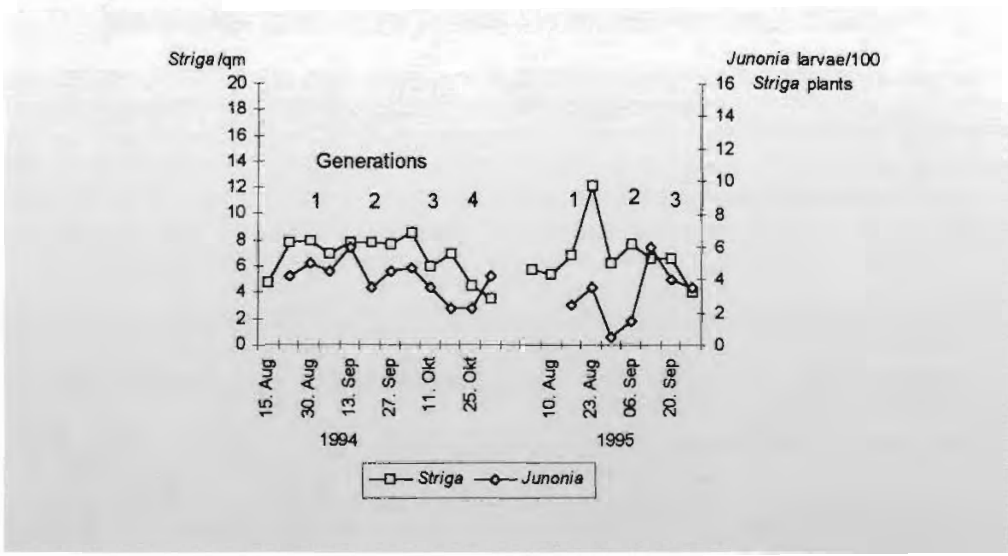

Figure 2

Population dynamics of *Junonia orithya* in northern Ghana in 1994 and 1995 in eight and two farmers' fields, respectively.

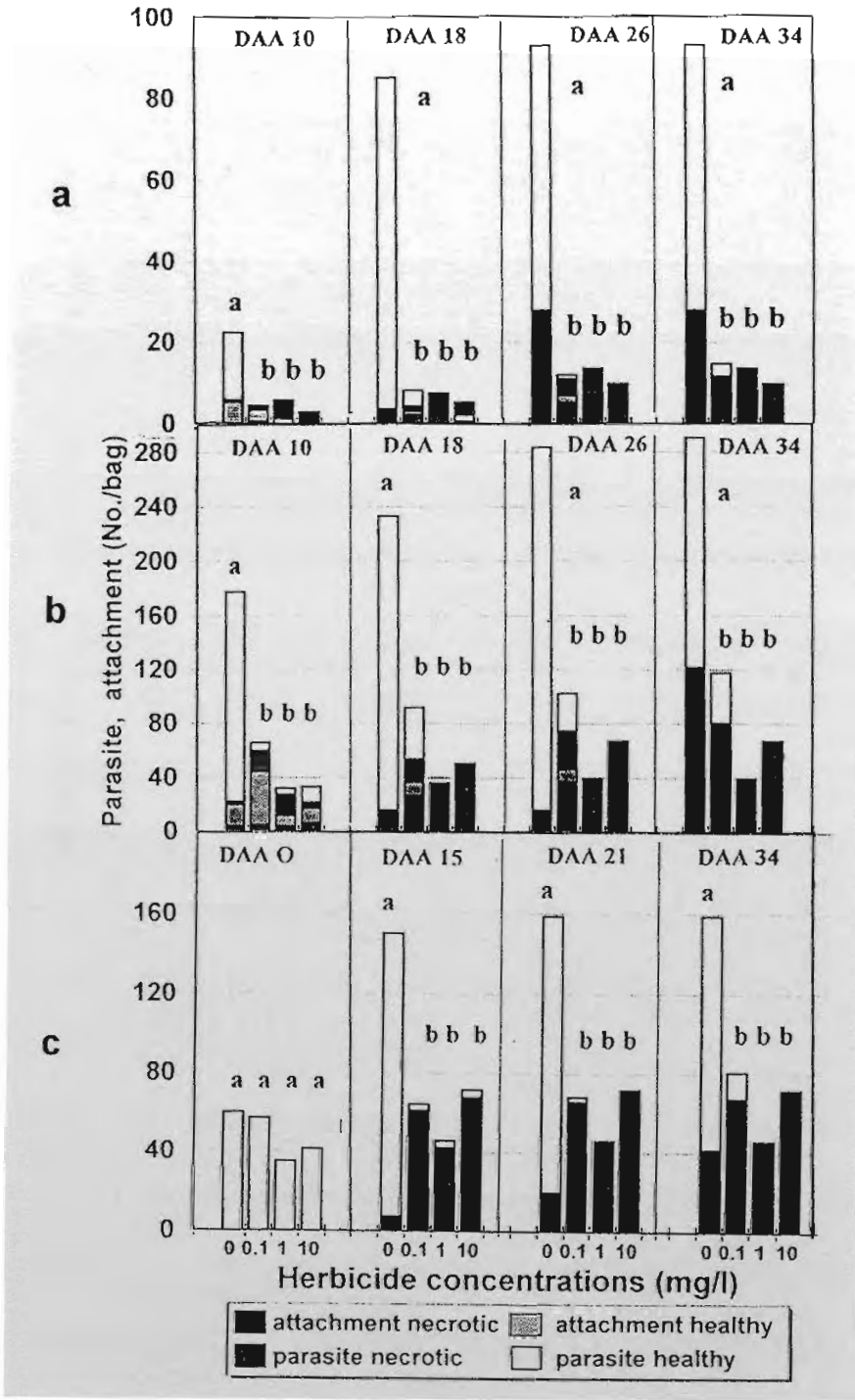

Figure 1

Effect of chlorsulfuron on *O. aegyptiaca* development in PE bags Applied at preconditioning (a), germination (b) and tubercles (c) stages. Attachment (radicle penetration into root epidermis), parasites (tubercles, spiders, stems). DAA-days after application. Bars in each observation period, followed by a different letter days significantly at *P=0.05* according to Duncan's multiple range test.

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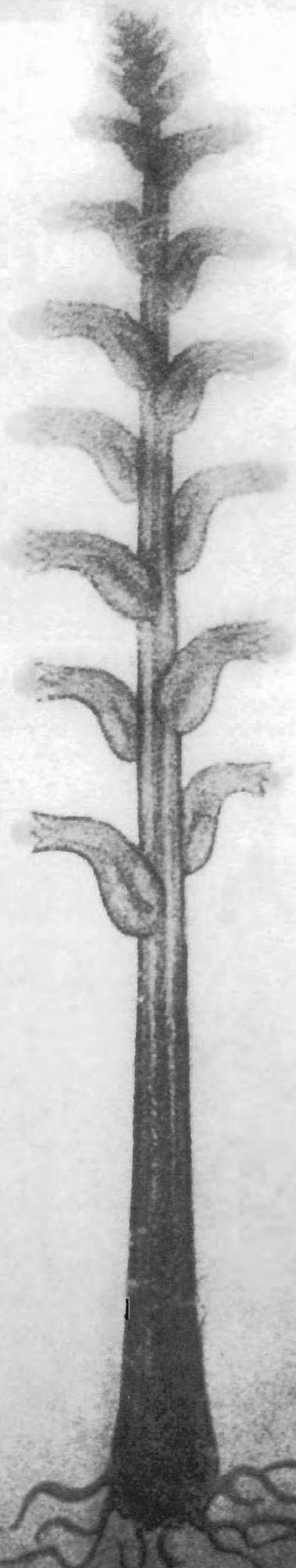

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April 16-17-18, 1996

Families and Tribes

Annexes

Genera and Species

Introduction

Several fungi have been reported as pathogenic to *Orobanche* spp. (Talskh Yan and Grigoryn, 1978). *Rhizoctonia solani* isolated from necrotic stem of *Orobanche ramosa* resulted in 68% reduction in broomrape emergence and failure of the broomrape to produce mature seeds. In earlier studies, *Sclerotinia orobanche* and *Sphaerotheca fuligiea* (Poletaeva *et al.*, 1963) were isolated from different species of *Orobanche*. The present study was carried out in two seasons with the objectives of screening, isolating and identifying different fungal genera and species in connection to *Orobanche* spp. infestation in different geographical locations. The isolated agent were then tested for their pathogenicity on juvenile underground stages of *Orobanche* spp. in an *in vitro* test.

Material and Methods

Fungi screening in the soil rhizosphere

Five Egyptians governorates were chosen, El-Dakahlia, El-Beheira, Bani-Seuf and Assiut to represent North, Middle and South Egypt, because of the existence in them of natural infestation, different hosts and different *Orobanche* species. Samples were collected every two weeks, during winter seasons 1993-1994, from two locations in each governorate: (A) infested with *Orobanche* spp. and (B) uninfested. Samples were assayed for fungi isolation according to the method developed by Louw and Webly (1959). Total fungal count was followed according to Allen (1961) using Martin medium (Anonymous, 1978). Fungal colonies were counted after 5 and 7 days of incubation at 25°C. Fungi were isolated from solitary colonies and purified, then identified according to Gilman (1957) and Barnett and Hunter (1972). Pure fungal cultures were maintained on PDA slants medium (Anonymous, 1978) at 5°C for furthers studies.

In vitro pathogenicity test on *Orobanche* spp.:

Isolated pure cultures were divied into three groups according to geographical samples, i.e. North Egypt group (Beheira, Dakahlia and Gharbia governorates), Middle Egypt group (Bani-seuf governorate) and South Egypt group (Assiut governorate). Selected undamaged juvenile underground stage of *Orobanche* spp. was surface disinfected with 1% sodium hypochlorite solution, washed in sterilized water and dried gently with filter paper. Disinfected broomrape tissue was placed in petri dishes containing PDA medium inoculated with each one of the tested fungi. Petri-dishes were then incubated at 25°C for 7 days and examined. Three replicates were used for each fungal isolate and one as uninoculated control. Pathogenicity was determined (Kiraly et al., 1974) as high, moderate, weak and no infection, in respect to the percentage of damaged tissues area resulting from *Orobanche* reaction to different fungi inoculated onto the medium.

Results

Isolated fungi, from rhizosphere of different plants were identified as species of *Alternaria, Aspergillus, Cladosporium, Fusarium, Penicillum, Mucor, Rhizopus, Trichoderma, Trichothecium, Synchetrium* (Phycomycetes) in addition to other unknown fungi. The average frequency of these fungi is presented in Table 1. Quantitative and qualitative differences were observed between uninfected and infected samples. Species of *Aspergillus, Fusarium* and *Penicillum* were frequent in host rhizosphere under *Orobanche* infested conditions. The rest of the isolated fungi varied in the rhizosphere samples according to governorate, geographical location, and host.

The previous isolated fungi were tested for their ability to attack broomrape tissues *in vitro*. The degree of infected tissues was recorded according to the damaged area as follows: (1) high infection (damaged area >50%), (2) moderate infection

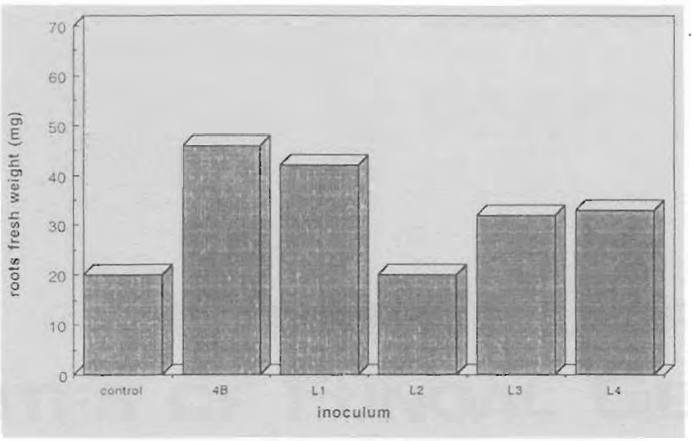

Figure 4

Effect of bacteria on the weight of *Sorghum* fresh roots.

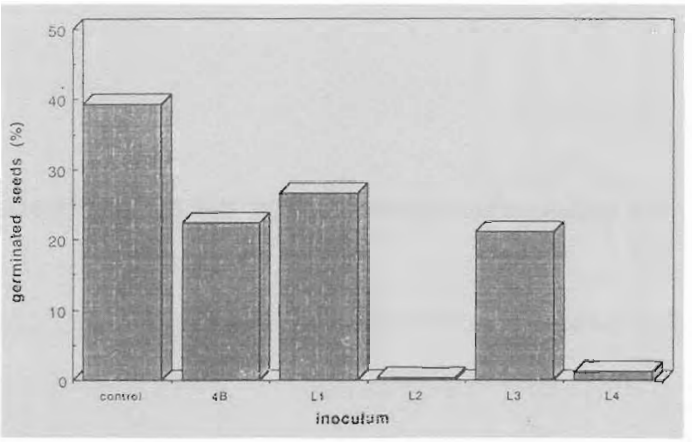

Figure 5

Effect of Bacteria on *Striga* seeds germination.

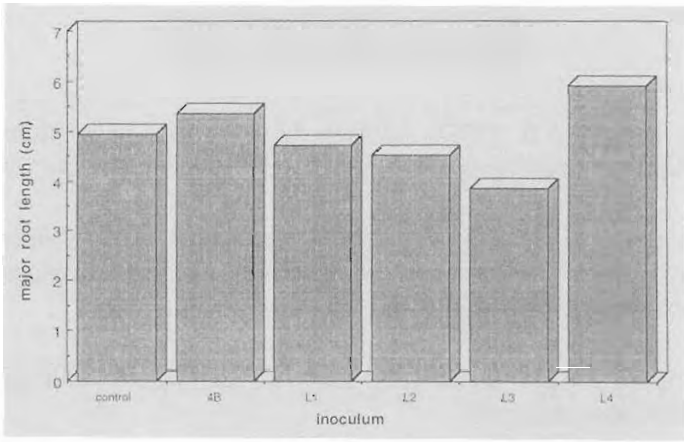

Figure 1

Effect of bacteria on the length of *Sorghum* main root.

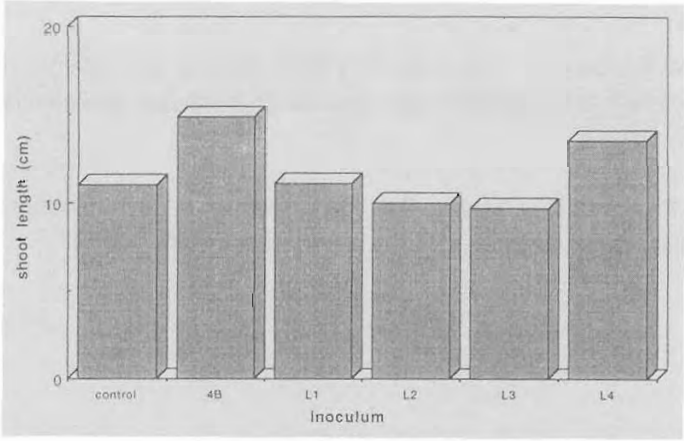

Figure 2

Effect of bacteria on the length of *Sorghum* shoot.

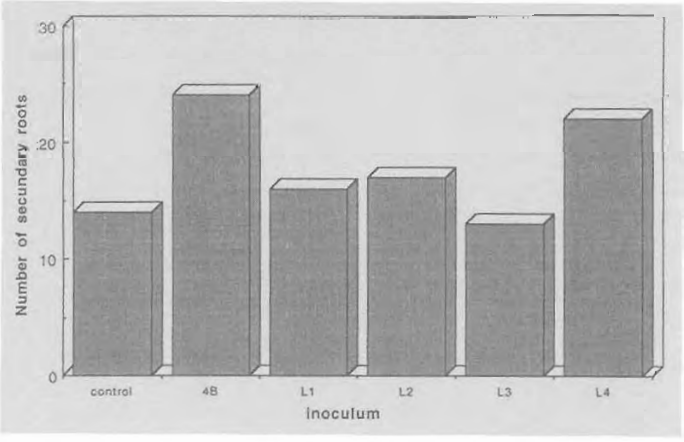

Figure 3

Effect of bacteria on the number of *Sorghum* secondary roots.

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length of shoot and of major root were measured and secondary roots were counted. Bacteria were extracted from roots by blending for 1.5 min in 10 ml of sterile 0.8% NaCl with an homogeneizer (Ultraturax). Appropriate dilutions of the extract were spread onto Nfb medium supplemented with cycloheximide (200 $mg.ml^{-1}$) to prevent fungal growth. Bacterial colonies were counted after 4 days of incubation at 28°C.

Assay on Striga germination: Two day old sorghum plantlets (see above) were placed on Nfb-agar Petri dishes, with or without bacteria (strain L_1, L_2, L_3 or L_4). Preconditionned disks covered with seeds of *Striga*, were then placed on roots of *Sorghum*. Controls without *Striga*, without *Sorghum* or without bacteria were prepared. Germination of seeds of *Striga* was scored after 3 days, under a binocular lens. Each experiment was repeated five times.

Statistical analysis of data: Analysis of variance was performed to test differences between each condition of Sorghum inoculation and *Striga* germination. Differences were considered to be significant at the $p<0.05$ level.

Results

Effect of inoculation of bacteria on Sorghum growth

Results are shown in Fig. 1, 2, 3 and 4. The strain noted as 4B, used as PGPR standard, is an *Azospirillum lipoferum* strain isolated from rice rhizosphere (Thomas-Beauzon *et al.*, 1983).

From these results, a significant PGPR effect was observed by inoculation of the *A. brasilense* strain L4: increase of the root bulk, due to an important increase of the number of secondary roots (compared to control, Fig. 3) and also increase in shoot length (Fig. 2).

Another strain, *A.brasilense* L1, also caused a significant effect on the fresh weight of roots (Fig.4). Srandard strain 4B showed also a significant positive effect on fresh weight of roots, number of secondary roots and shoot length.

The presence of the inoculated bacteria on roots, was verified. All strains were present (10^6 to10^7 cells by g of fresh root) at the end of the experiment.

Effect on germination of *Striga*

A. brasilense L_2 and L_4 were the only strains to inhibit completely germination of *Striga* compared to control without bacteria (Fig. 5). *A. brasilense* L_3 also had a significant inhibitor effect on the germination of *Striga* compared to control without bacteria. Nonetheless, this effect was much lower than that produced by strains L_2 and L_4.

Discussion

These results showed that an *A. brasilense* strain (L4) isolated from a soil under sorghum was able, *in vitro*, both to inhibit germination of *Striga* and to promote growth of Sorghum. Another strain (L2) showed the same inhibitor effect on the parasite seed germination but failed to increase growth of the cereal. These strains were isolated from an uncontamined area in the field. Therefore we suppose that under natural conditions, these bacteria have a major impact on both parasite germination and cereal growth.

Today, PGPR bacteria are used in several countries as inoculants in agronomy. Their beneficial effects on plant growth include a significant reduction (about 30%) of nitrogen fertilization. *A. brasilense* L4 could be a good candidate as an inoculant for growth promotion and fertilizers economies and, at the same time, it would also be a good tool to reduce parasitic infestation; a double benefit for *Striga* contaminated and nutrient deficient soils.

Introduction

Bacteria of the genus *Azospirillum* are widespread in nature (Döbereiner and Pedrosa, 1987) and usually found closely associated with important crop cereals like sorghum, rice and maize (Tyler *et al.*, 1979). These microorganisms have been used as potential plant growth promoting rhizobacteria (PGPR) (see for example the review of Glick, 1994) for many cereal crops. On the other hand, the parasitic weeds *Striga* spp. also colonize cereals and therefore have the same plant host specificity.

The hypothesis was that a possible competition to colonize host plant existed between bacteria and parasite, and that bacteria could control the latter. First, to respect the host-microorganism specificity, which is important to obtain a beneficial effect, bacteria of the genus *Azospirillum* have been isolated from soil under sorghum, both from *Striga hermonthica* contamined and uncontamined areas (Kabir. data non shown). From uncontamined soil, four *Azospirillum* strains have been isolated, using a specific method, and characterized by oligonucleotide probes (Kabir *et al.*, 1995) as *A. brasilense* strains.

In this work, we describe the results about the effects of these bacterial strains on both *Striga* germination and plant growth.

Materials and Methods

Bacterial strains and growth conditions: *A. brasilense* strains L_1, L_2, L_3 and L_4 have been isolated from a soil under sorghum (in Mali) in an area uncontamined by *Striga*. These strains were grown on Tryptone Yeast extract (TY) medium: ($g.l^{-1}$) yeast extract, 3g; bactotryptone, 5g, at 28°C. For preparation of inocula for PGPR testing, bacteria at the end of Log phase cultures were washed three times with sterile 0.8% NaCl, sedimented by centrifugation for 20 min at 7000 g, at 4°C, and then resuspended in a volume of sterile 0.8% NaCl adjusted to obtain a concentration of 10^8 cells.ml^{-1} (OD_{540nm} = 0.8). For *Striga* germination assays, bacteria were pre-inoculated on TY medium and, after one day, inoculated in Nitrogen free broth (Nfb) (Krieg and Döbereiner, 1984) and incubated at 28°C, in the dark, with agitation. When the culture reached an OD of 0.6 (10^6 cells. ml^{-1}), 250 µl were spread on Nfb- Agar plates (Agar 15 g/liter).

Plants seeds, sterilization and germination: Seeds of *Sorghum vulgare* (cv Tiemarifing), susceptible to *Striga*, were surface sterilized, twice in 3%(v/v) hydrogen peroxide for 20 min, washed three times with sterile water, and once with 1%(w/v) calcium hypochlorite, for 2 hours. After washing three times with sterile water, seeds were plated on a Petri dish containing sterile 0.8 % agar and allowed to germinate at 28°C, in the dark, for 2 days.

Striga hermonthica seeds (from Burkina Faso) were sifted and surface sterilized (Ouedraogo, 1995) in 70%(v/v) ethanol for 2 min, then, in 1% (w/v) calcium hypochlorite, with three drops of "Tween 20", for 10 min. Seeds were rinced with sterile water and placed on 6 mm diameter filter paper disks that were themselves placed on moistened Whatman GF/A papers in Petri dishes. Closed Petri dishes placed in the dark, at 31C, for at least 14 days.

Assay of bacterial inoculation on sorghum: Microcosm experiments (Steinberg *et al.*, 1989) were done using 10 ml syringes filled with 10 g of sterile Fontainebleau sand. One 2 day old axenic *sorghum* plantlet was introduced into each microcosm. Inoculation of bacteria was carried out by adding 3 ml of each bacterial inoculum, prepared as described before. This was repeated five times for each condition of inoculation. Five microcosms were inoculated with 3 ml of sterile 0.8% NaCl as control. All microcosms were then placed into a growth chamber under the following conditions : photoperiod 16-8; temperature, 28°C during the day and 19°C during the night.

Twenty-one days after inoculation, plantlets were removed from the sand, fresh weight of roots,

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INHIBITION OF *Striga* SEED GERMINATION BY BACTERIA ISOLATED FROM SOIL UNDER SORGHUM FIELD

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ABSTRACT

Striga spp. are obligate parasitic weeds of tropical cereals and generally have the same host plant as the rhizosphere bacteria of the genus *Azospirillum*. Four strains of *Azospirillum brasilense* isolated from soil of a sorghum field, have been tested for their effect on germination of *Striga* seeds. Two out of four strains assayed significantly inhibited germination of parasite. Moreover, one strain showed a plant growth promoting effect (PGPR effect).

Additionnal key words: biological control, plant-bacteria interaction.

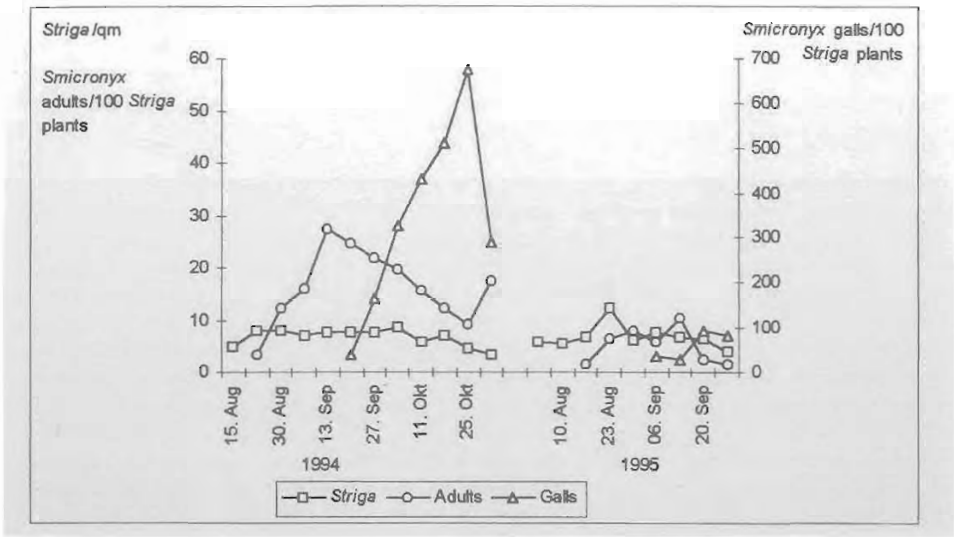

Figure 1

Population dynamics of *Smicronyx* spp. in northern Ghana in 1994 and 1995 in eight and two farmers' fields, respectively.

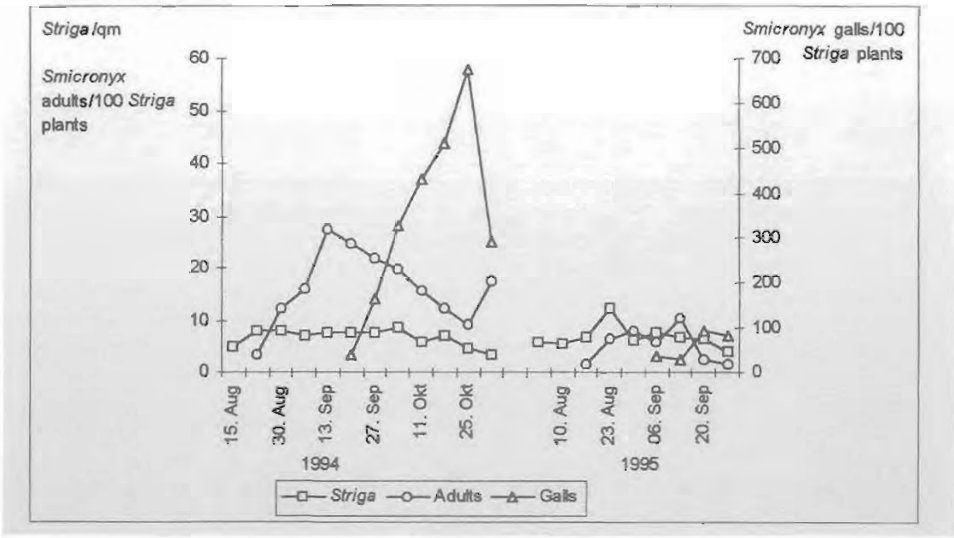

Figure 2

Population dynamics of *Junonia orithya* in northern Ghana in 1994 and 1995 in eight and two farmers' fields, respectively.

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Results

Population dynamics and influence of *Smicronyx* on *Striga hermonthica*

In northern Ghana two different species of the genus *Smicronyx* were collected from *S. hermonthica: Smicronyx umbrinus* and *S. guineanus* Voss. However, no differentiation was made between both species in our following preliminary evaluation. The field observations revealed that the first *Smicronyx* adults apppeared mid of August on *S. hermonthica.* After copulation eggs were laid into the inflorescence of *S. hermonthica.* This induces gall-forming of the seed capsules, in which the larvae develop. The first galls were observed mid of September (Fig. 1) with the highest infestation determined mid to end of October with 676 galls per 100 *Striga* plants on average. At the same time the larvae started to leave their galls for pupation.

These observations correspond to the pot experiments where the first larvae left their galls also in mid of October to enter the soil. After one week the larvae started to form earthen cells for pupation. Some of the *Smicronyx* adults hatched already in December, but most of them remain as pupae until the first week of August. Since the development of *Smicronyx* larvae is closely related to the capsule developement of *S. hermonthica,* it is assumed that only one generation is developed per year.

Furtheron, the pot experiments revealed, that one *Smicronyx* female caused on average the development of 14 galls on 3 *Striga* plants on average, equalling to about 14 larvae. The egg-laying capacity as well as the egg mortality could not be determined since the eggs layed were not found on the *Striga* plants.

In the eight fields surveyed, on an average 22.5% of the *S. hermonthica* plants were infested with *Smicronyx* galls (Table 1). The mean infestation intensity of one *S. hermonthica* plant (number of seed capsules per *Striga* plant transformed into galls) was 77.3%. Only one Smicronyx larva developed per gall, whereby no *Striga* seed development was possible. The calculation on the impact of *Smicronyx* on the seed production of *S. hermonthica* in the field based on the data collected in 1994 indicated a reduction of 17.4% under natural conditions (Table 2).

Population dynamics and infestation of *Junonia orithya* and its host range

Field observations indicated, that the adults of J. *orithya* appear in the beginning of July and the first larvae were found on *S. hermonthica* in mid of August (Fig. 2). In the eight fields surveyed. on an average 11.3% of the *S. hermonthica* plants were infested with larvae of *J. orithya* with six larvae on average per 100 infested plants (Table 1).

Further, it could be observed, that pupation takes place on maize, sorghum or millet as a suspended pupa hanging upside down. In the laboratory, pupae were mainly found on top of the insectaries. The duration of the development of *J. orithya* from pupa to adult was 8 days on average. It can be assumed that 3 to 4 generations develop per year since in an intervall of 2 to 3 weeks a remarkable high number of very tiny larvae of the first and second instar were found on *S. hermonthica.* However, this could not be proved in the laboratory, as in the insectaries no egg-laying took place even though the adults of *J. orithya* lived up to 20 days in the insectaries.

The non-choice test proved, that none of the food crops cultivated in northern Ghana is attacked by *Junonia* larvae.

Discussion

Smicronyx spp. and *Junonia orithya* are found as herbivores of *Striga hermonthica* in the savanna zone of northern Ghana. For both, further

Impact of *Smicronyx* spp. on the reproduction of Striga hermonthica

The larvae of *Smicronyx* spp. inhibit the production of *S. hermonthica* seeds through their feeding habit in seed capsules. In order to estimate this impact under natural conditions the data basis of the eight fields surveyed in 1994 was used for calculations (Tab.1) and the mean values of the *S. hermonthica* infestation (plants per m^2), the percentage of *S. hermonthica* plants infested with *Smicronyx* galls and the number of galls calculated as the percentage of the total number of seed capsules per *S. hermonthica* plant were determined. In addition, 200 galls were collected and opened with a scalpel to determine the number of larvae and viable seeds per gall. With regard to investigations carried out by Sprich (1994) on the number of viable seeds produced per *Striga* plant the reduction of the seed production due to the infestation of *Smicronyx* spp. was assessed.

Preliminary autecological investigations into the rearing of *Smicronyx* spp. and *J. orithya*

In order to consider herbivores in biological control programmes rearing methods have to be established. With regard to *Smicronyx* spp. and *J. orithya* no rearing methods have been described in the literature. Therefore, some preliminary autecological studies have been carried out.

Smicronyx adults were removed from *S. hermonthica* plants in the field by an exhaustor (suction method). Since *Striga* spp. are the only host plants, in the growing seasons 1994 and 1995 respectively, six pots prepared with *S. hermonthica* seeds were planted with sorghum. In order to determine the reproduction rate per female, after the emergence of *S. hermonthica* one pair of *S. umbrinus* (male and female) was placed in each pot and the pot was surrounded with a stiff plastic foil and covered with nylon gauze. The number of galls produced per female was recorded as well as the time when the first larvae left their galls. In addition, for further information on the life cycle of *Smicronyx* spp. larvae were collected also by removal of fully developed galls from *Striga* plants in the field, which were put in containers with a 10 cm wet soil layer. The larvae left the galls and pupated in earthen cells in the soil, where they could be sieved out and recorded.

Junonia adults were either caught in the field with an insect net or the larvae were collected from Striga plants by hand and put in cages made out of wood and mosquito wire. The larvae were regularly fed with fresh *Striga* plants from fields until pupation. In each insectary a potted *Striga* plant was situated and sugar water was provided to the adults for food to stimulate egg-laying.

Studies on the host range of *Junonia orithya* larvae

In order to prove whether the polyphagous *Junonia* larvae attack food crops cultivated in northern Ghana non-choice tests were carried out with food crops of northern Ghana. Therefore, leaves of sorghum (*Sorghum bicolor* (L.) Moench), maize (*Zea mays* L.), pearl millet (*Pennisetum americanum* (L.) Leeke), yam (*Dioscorea rotundata* L.), cassava (*Manihot esculenta* Crantz), okra (*Abelmoschus esculentus* L.), cotton (*Gossypium* spp.), cowpea (*Vigna unguiculata* L.), soybeans (*Glycine max* L.), groundnut (*Arachis hypogaea* L.), congo jute (*Urena lobata* L.), kenaf (*Hibiscus cannabinus* L.), tomato (*Lycopersicon esculentum* L.) and chili pepper (*Capsicum annuum* L.) were collected in the field and offered to ten Junonia larvae of the third and fourth instar. After 24 hours it was recorded whether they feed on them or not. The experiments were done with three replications.

Introduction

Most of *Striga* spp. damage to the host occurs before emergence of the parasite from the soil, thus making soil-borne pathogens which reduce *Striga* spp. seed germination potentially valuable *Striga* management components. The prevalent soil-borne pathogen isolated from diseased *Striga* plants belong to the genus *Fusarium* (Abbasher and Sauerborn, 1992; Kirk, 1993; Abbasher *et al.*, 1995; Ciotola, 1995). Four species: *Fusarium nygamai, F. semitectum* var. *majus, F. oxysporum,* and *F. solani* were all found to reduce the emergence of *S. hermonthica* when incorporated into soil preplanting. All *S. hermonthica* growth stages from unemerged seedlings through mature, flowering *Striga* were attacked and killed by these fungi. The objective of this study was to investigate the ability of five *Fusarium* isolates (one isolate of each: *F. nygamai, F. emitectum* var. *majus, F. solani,* and two isolates of *F. oxysporum*) to inhibit seed germination of three *Striga* species: *S. hermonthica* (sorghum and millet strains), *S. gesnerioides* and *S. asiatica.*

Material and Methods

Five *Fusarium* species were employed in the present study: *F. nygamai* was isolated from S. *hermonthica* plants in the Blue Nile region, Sudan, in 1989; *F. oxysporum* (isolates one and two) and *F. solani* were obtained from diseased *S. hermonthica* collected in Northern Ghana in 1992; and *F. semitectum* var. *majus* was cultured from a wilting *S. asiatica* plant parasitizing maize in Madagascar in 1993. *F. semitectum* var. *majus* was investigated only in the root chamber study.

Seed sources. Seeds of *S. hermonthica* (millet and sorghum strains) were collected in 1991 in Bengou (Niger); seeds of *S. gesnerioides* from cowpea were provided by G. H. Schmelzer (DFPV-Niger) from her 1994 collection; and *S. asiatica* seeds collected in 1989 from maize in Kenya, were supplied by Dr. J. Ransom. Root exudates of the highly susceptible Bengou millet and sorghum local landraces, the cowpea variety (Sadoré local), and the maize variety (Makka local) were used to stimulate germination of *S. hermonthica*, *S. gesnerioides*, and *S. asiatica*, respectively.

Seed disinfection. *Striga* seeds were placed into 50 ml Erlenmeyer flasks and were washed 3 times in 10 ml sterile deionized water to which 3 drops of 10 % Tween 20 had been added. The washed seeds were surface disinfested in 10 % Sporicidin® solution (Sporicidin International, Rockville, MD, SA) by sonicating for 3 minutes with occasional swirling after which seeds were rinsed 3 times in distilled water.

In the first experiment *Striga* seeds were preconditioned for two weeks at 28C in either 10 ml of sterile distilled H_2O (control) or 10 ml of suspension containing fungal hyphae and approximately 3 x 10^5 spores/ml. In a second set of experiments, treatments were identical except that fungal suspensions were amended with 10 % sucrose (w/v). In a third set of experiments, the effect of concentrated spore suspensions (1 x 10^6 spores/ml) on germination of *S. hermonthica* seed was investigated.

Seed germination. *Striga* seeds were stimulated to germinate by one of two techniques: a) exposure for 24 hr to aqueous root exudates collected from 2-week-old host seedlings (sorghum, pearl millet or cowpea); or b) by placing pearl millet and sorghum germlings in an agar gel assay described by Hess *et al.* (1992).

Preparation of Petri dishes. For the aqueous root exudate assay, small (1 cm diam.) glass microfiber filter paper discs (Whatman GF/A) were placed onto 2 filter paper disks (Whatman No.1) in 9 cm Petri dishes. Subsequently 50-200 preconditioned *Striga* seeds were pipetted onto each disc. Petri dish preparation followed the methodology of Hess *et al.*, 1992.

Aqueous root exudate. About 20 seeds of each host crop were sown in small plastic pots (10 x 10 x 10 cm) filled with washed sand in the glasshouse.

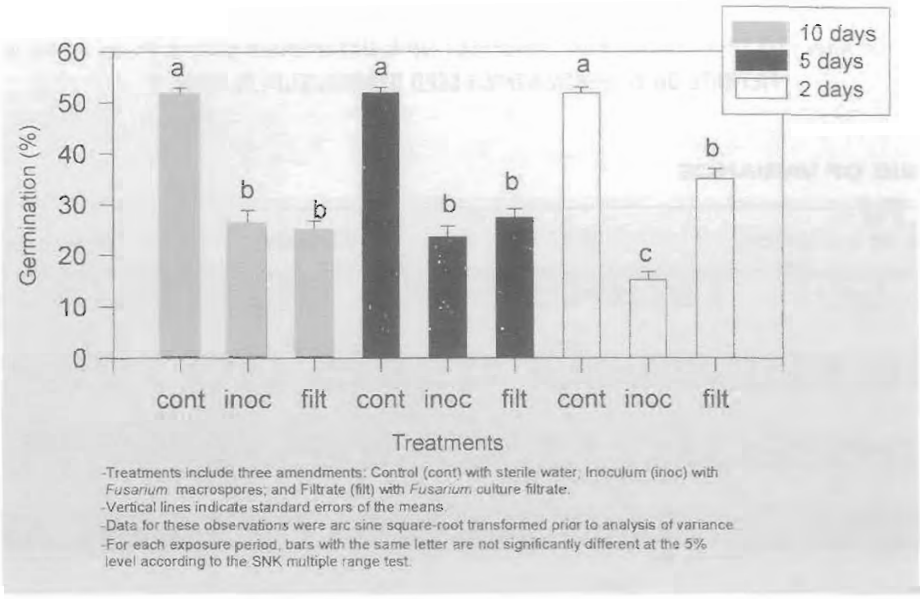

Figure 1

Germination of *Striga hermonthica* exposed to *Fusarium oxysporum* macrospore or crude filtrate solutions during preconditioning (first experiment).

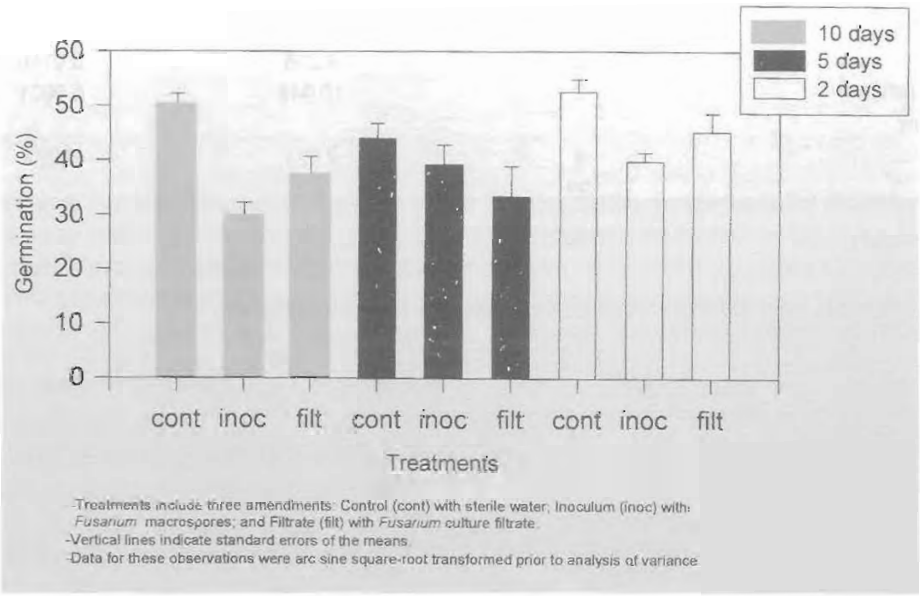

Figure 2

Germination of *Striga hermonthica* exposed to *Fusarium oxysporum* macrospore or crude filtrate solutions during preconditioning (second experiment).

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exudate. Since macrospores were not separated from their matrix before inoculation, the observed reduction in seed germination for this treatment could be attributed to the coupled effect of the spores and the matrix (concentrated crude filtrate).

The decline in germination capacity following exposure to either crude filtrate or fungal propagules suggests that *S. hermonthica* seed structures were possibly altered in such a way as to render the sorghum germination stimulant ineffective in triggering germination. The structural modifications to the seed coat could be of a physical and/or of a chemical nature, thereby blocking the chemical binding of the stimulant to the seed. Seed infection by *F. oxysporum* spores could also account for reductions in *Striga* seed germination. In the case of *Orobanche cumana*, the black pigments of the seed coat are thought to prevent fungal infection of the seed (Krenner, 1958). Bedi & Donchev (1991) reported that fungal infection of *O. cumana* by *F. oxysporum* f. sp. *orthoceras* occurred only on the soft tissue of the first radicle. Similarly, Abbasher & Sauerborn (1992) noted the infection of radicles of *S. hermonthica* germlings inoculated with *F. nygamai*. Moreover, these authors observed large reductions in the number of germinating seeds after inoculation in root chambers. In the latter, *Striga hermonthica* seeds were preconditioned prior the inoculation.

Variability in results for exposure lengths in the two trials makes it difficult to determine the appropriate exposure time required for optimal *Striga* germination suppression. However, results do indicate that *S. hermonthica* seeds are susceptible to *F. oxysporum* M12-4A at each of the three duration periods examined during the preconditioning phase.

B) Electron microscopy (TEM) observations of *S. hermonthica* seeds inoculated with *F.oxysporum*

Observation of ultrathin sections of samples inoculated with *F. oxysporum* M12-4A revealed the presence of the fungus within seed structures. These preliminary results, might explain partly the reduction in seed germination through the capacity of this *F. oxysporum* isolate to penetrate and infect seed tissues. Abbasher (1994) reported *F. nygamai* within inoculated seeds of *S. hermonthica*. Further research is needed and will focus on the morphological changes occurring to *S. hermonthica* seed and seed coat in the presence of *F. oxysporum* macrospores and crude filtrate as well as on the mode of penetration of the fungus.

Acknowledgments

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by germinating surface sterilized sorghum seeds and placing them in test tubes filled with a 28 ppm Drew & Saker solution (Drew & Saker, 1986). After 7 days, the root exudate collected in the growth media was filtered through a 0.45µ Whatman® filter.

iv) Germination assessment After the 10 day period, *S. hermonthica* seeds from all treatments were washed with sterile water and inserted into wells of a microtitre plate (Plate 1). Each well received 50-100 seeds from dishes of the various treatments as well as a volume of 0,1 ml of germination stimulant extracted from sorghum roots. Microtitre plates were wrapped in aluminum foil and placed in an incubator set at 30°C. Each treatment consisted of 12 replicates. Seed germination was assessed after 24 hours. The experiment was repeated once.

v) Statistical analysis. Data were expressed as percent germination and were arc sine square-root transformed before statistical analysis (MANOVA). Means were separated using the Neuman-Keuls (NK) multiple comparison test.

B) Electron microscopy (TEM) observations of *S. hermonthica* seeds inoculated with *F. oxysporum*

Striga seeds of each treatment were embedded in agarose blocks prior to the fixation of the material. The procedure used to prepare samples for TEM (transmittance electron microscopy) was described by Olivier *et al.* (1991). Fixation of the samples was carried out using the double fixative technique with glutaraldehyde (3%) and osmium tetraoxide (1%) with a cacodylate buffer. Following the dehydration of the material in a graded series of ethanol, infiltration was performed using a resin composed of Epon 812, DDSA, NMA and DMP 30 and polymerization was activated. After hardening, samples were cut in thin (1 µm) and ultrathin (0,1 µm) sections using an ultramicrotome.

Results and Discussion

A) Quantification of the effect of *F. oxysporum* M12-4A macrospores and crude filtrate on the germination of *S. hermonthica*

Given the results of the analysis of variance where we found a significant effect of the trial factor, each experiment was treated separately.

There was a sharp reduction in germination for seeds treated with either macrospores or crude filtrate from *F. oxysporum* for all three exposure periods (10, 5, 2 days) (Figure 1). Crude filtrate and macrospore treatments for each exposure period were all significantly different from the control (Figure 1, Table 1). The lowest germination (15%) was observed for the 2-day exposure treatment using a fresh *Fusarium* macrospore solution. This treatment accounted for a 71% reduction in germination compared with controls. In this treatment, seeds were immersed in water for 8 days prior to inoculation. This soaking period could help soften the seed teguments thereby making seeds more permeable and/or more easily penetrable by the fungus and its filtrate. Valance (1950) suggested that the aleurone layers of the *S. hermonthica* seed become more permeable during preconditioning. When the experiment was repeated, *Fusarium* treatments (crude filtrate and spores) resulted in similar germination levels for each exposure period (Figure 2, Table 2). Although the reduction in gemination recorded in trial 2 was lower than in trial 1, significant reductions in *S. hermonthica* seed germination were, nonetheless, obtained. The highest suppression in germination (41%) was found for seeds incubated with macrospores for a 10 day period (Figure 2, Plate 2 a, b). Signs of radicle infection were also recorded for samples inoculated with *F. oxysporum* M12-4A (Plate 3). The possibility that variation in germination rates were due to changes in the microenvironment of seeds is unlikely since seeds for all treatments were rinsed with sterile water prior to their stimulation with the sorghum root

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IMPACT OF *Fusarium Oxysporum* ISOLATE M12-4A UPON SEED GERMINATION OF *Striga hermonthica* IN VITRO

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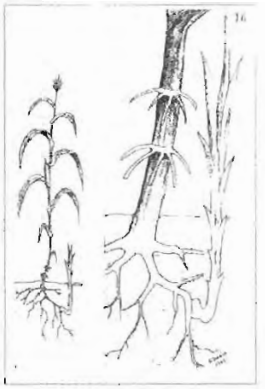
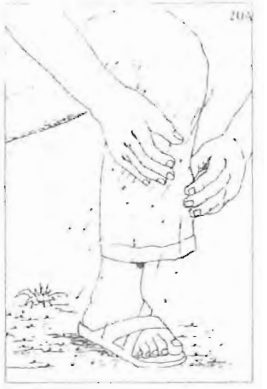
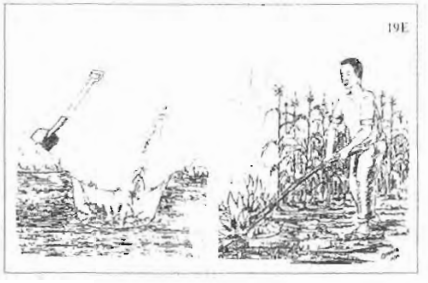
ABSTRACT

Isolate M12-4A of *Fusarium oxysporum* from Farabana, Mali is under development as a bioherbicide for the control of *Striga hermonthica* in West Africa. *F. oxysporum* acts at early stages of the development of *S. hermonthica*, eliminates emergence of the parasite from pots in a controlled environment and reduces germination and attachment in root chambers. An in vitro experiment was used to measure the impact of *F. oxysporum* on *S. hermonthica* germination. During a 10 day preconditioning phase, *S. hermonthica* seeds were exposed for periods of 2, 5 or 10 days to 5 ml of a macrospore suspension (107 spore ml^{-1}), to a crude filtrate from which macrospores had been removed. At the end of the preconditioning phase, fresh root exudates of sorghum were added to *S. hermonthica* seeds to stimulate germination. Germination was assessed after 24 h. All treatments significantly reduced *S. hermonthica* germination with the greatest suppression reaching 71% with the 2 day exposure to macrospores. When the experiment was repeated, the greatest suppression (41%) was observed with a 10 day exposure to macrospores. These results demonstrate that the activity of *F. oxysporum* can be attributed, at least in part, to a direct inhibition of seed germination of *S. hermonthica*.

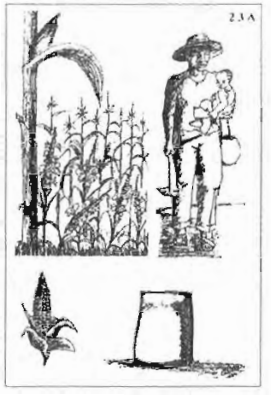






Legend:

9b: Seed dispersal by men, tools and animals
9c: Seed dispersal by crop seeds
15: Subterranean *Striga*
16: Emerging *Striga*
18d: Crop rotation: soybean
19: Burying/Burning
20a: Preventive methods: cleaning
21b: Organic manure: collecting
23a-c: Final pictures (long-term approach)



Introduction

Striga hermonthica (Del.) Benth. presents a complex problem in cereal cropping in northern Ghana. Within the supra-regional project "Ecology and Management of Parasitic Weeds", which has been supported by the Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) and the University of Hohenheim, different farming systems in the Guinea and Sudan savanna of northern Ghana have been defined as pilot regions for the development and implementation of adapted control strategies. Since 1988, in co-operation with the Savanna Agricultural Research Institute (SARI), Nyankpala, several research projects have been realised with regard to the use of different cultural practices (e.g. crop rotation, cover crops) and biological control (Vogt, 1993; Sprich, 1994; Abbasher *et al.*, 1995; Kroschel *et al.*, 1995; Jost *et al.*, 1996). In addition, analyses of the farming systems were performed in order to recognise limitations as well as the adoption capacity of control methods (Runge-Metzger, 1993; Kroschel and Sauerborn, 1996; Kroschel *et al.*, 1996). An integrated *Striga* control strategy for northern Ghana was derived from these studies presented in a training manual and discussed with extension staff in training courses (Kroschel and Sauerborn, 1993).

In many African countries the training and visit (T&V) system has been introduced and modified. The "Unified Extension System" in Ghana is also based on this approach. Within the existing framework FLS (front line staff) have been unable to effectively support farmers to combat *Striga*. Appropriate extension materials are lacking in most countries. The need for practical training of FLS was revealed during surveys (Sprich, 1994) and through evaluations at training courses (Osterburg, 1995) which indicated that the knowledge about the biology and control of *Striga* is poor. However, knowledge should be made available to farmers and possible solutions should be based on the individual potential of farmers. A participatory approach for the extension of *Striga* control measures is therefore necessary to tailor such strategies to farmers' needs. A group extension programme supported by visual aids, was thought to meet the requirements for explaining the complexity of *Striga* biology and the integrated long-term control approach needed for effective *Striga* management. The developed "*Striga* control programme" will be presented and experiences gained will be discussed within this paper.

Materials and Methods

In co-operation with the Ghanaian German Agricultural Extension Project (GGAEP), the Ministry of Food and Agriculture, Tamale, and two non-governmental organisations (Association of Church Development Projects (ACDEP) and Action Aid) an action research programme was initiated (Hummler, 1995). Pilot sites in the highly infested East Mamprusi, Tolon-Kumbungu and Savelugu districts were chosen. Based on the experiences with and related to the GRAAP (Groupe de recherche et d'appui pour l'autopromotion paysanne) methodology and the CFSME-approach (Conscientisation, Formation, Stimulation, Moyens, Evaluation), two teams of extension agents were trained and visual materials were developed, as were series of 41 hand coloured pictures, which could be used by extension staff with farmers groups (Hoffmann, 1991).

First, the two teams of extension staff discussed and decided on relevant information to be included in the extension programme. These results were presented to each other and discussed in a plenary session. After deciding on the messages the pictures should contain, they were transformed into written instructions and sketches for an artist well experienced in drawings of rural life.

The second step was to evaluate the first set of the picture series together with the extension staff and to check whether all necessary information has been included in the pictures and if the pictures are easily comprehensible. Necessary changes were discussed, preferably in the presence of the artist.

Thirdly, the first series of black and white material was used in a pre-test in ten selected villages. This

Introduction

In West Africa, more than 20 million hectares of cereal crops are infested with *Striga* (Lagoke, *et al.*, 1991). A recent study in Mali indicated that on 27% of the area infested with parasitic weeds, 86% is infested with *Striga hermonthica* (Del.) Benth. (ICRISAT, 1992). In southern Mali, where cereals are the staple food crop, *Striga* causes yield reductions of up to 50% (Sanogo *et al.*, 1994); additional losses are caused when farmers change their production strategies in response to heavy infestations (Kim, 1991).

Although many *Striga* control technologies have been developed, their adoption remains low (Kim, 1991). Most of the proposed technologies are labour and capital intensive, and have seldom been developed or assessed under local (African) farming conditions using a systems perspective. Consequently, farmers' opinions, knowledge and practices were insufficiently taken into account and the proposed technologies did not meet farmers' conditions (Chambers, 1993).

The Sikasso FSR team of the Malian agricultural research institute (IER), in collaboration with ICRISAT-Mali, has followed a participatory approach in dealing with the *Striga* problem, thereby complementing the ongoing experiment station research.

Materials and Methods

The study areas

The studies were conducted in two villages in the administrative region of Sikasso (71.790 km^2), in southern Mali: (1) *Try II* in the semi-arid zone in the north receiving an annual rainfall of 800 to 1000 mm; (2) *N'Golopéné* in the sub-humid zone in the south with a rainfall of 1200 mm or more. The landscapes are gently rolling with mostly poor soils that range from gravelly on the slopes to sandy and sandy loams in the lower areas. Further socio-economic details of the two villages are summarized in Table 1.

The action-research approach

The action-research was conducted by a multi-disciplinary team composed of an agricultural economist, agronomist and weed scientist as well as extension workers. The team worked in close collaboration with the farmers by using Participatory Rural Appraisal (PRA) techniques which encompassed "five successive steps" that were implemented in three days. The first three steps and the last take place at the village level; the fourth step is implemented at individual farms. The PRA techniques use interview guides that are prepared beforehand and are based on hypotheses about the factors and causes responsible for the *Striga* infestation (see also Table 1).

Step one: the village territory map. A village map is a schematic representation of the village territory as perceived by the farmers. Farmers mark on this map the various land units, as distinguished by (physical) characteristics, occupation and land use (Diarra *et al.*, 1995). The map is made by a group of villagers with some guidance from researchers, and serves to visualize: (1) the distribution of *Striga* over the village territory, and (2) the relations between the degree of *Striga* infestation and major territory characteristics such as soil types (often in relation to the toposequence) and land use.

Step two: the transect. The village territory and its major land units is crossed on foot by farmers and researchers to assess the level of *Striga* infestation and to analyze the relationships between toposequence land types, soil types, land use and *Striga* distribution. The transect constitutes a verification of the village territory map.

Step three: the farm classification. First, farmers identify the causes of *Striga* infestation and discuss possible control measures. Next they analyze the differences between farms as well as their causes. As a result management practices that explain differences between farms and the related, structural (socio-economic) factors are identified. Subsequently, farmers classify all the farms of the village into three or four classes according to their

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On-Farm Studies of *Striga* in South Mali: Contributions of a Farmer Participatory Approach

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ABSTRACT

In two regions of southern Mali, where cereals (maize, sorghum and millet) are the major staple crops, a farmer participatory and systems approach was used to investigate: the distribution of *Striga* over a village territory and within separate farm holdings, and the farmer perceptions about *Striga*, as well as the local techniques to control it.

The two regions have contrasting farming systems: in the sub-humid zone a shifting cultivation/fallow system is practised; in the semi-arid zone a permanent system. Both systems practise cotton-cereal rotations though the latter is more intensified.

Farmers knowledge and control practices differed substantially between the two regions and between farms: organic manure, urea applications and crop rotation are the *Striga* control practices cited in that order by farmers who use permanent systems; fallowing and early sowing are the preferred practices in the shifting agriculture. According to farmers toposequence land types and soil types are factors, that affect the occurrence of *Striga*, while run-off/erosion and cattle contribute to its spread. The much lower incidence of *Striga* in the permanent systems indicates the effectiveness of the actual farmer practices, and that a *Striga* problem tends to become less once intensified practices are adopted.

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In removing normal weeds, crop losses can be prevented in the same cropping season. In the case of a parasitic weed infestation, farmers cannot prevent yield losses even though they employ available control methods. Therefore, farmers have to practice a long-term approach which includes investments in technologies with uncertain short-term return.

Some economic principles can be deduced from assessments of the economics of *Striga* control by Runge-Metzger (1993) which might be important for farmers' decision alternatives. One *Striga* plant per field was taken as the control threshold since the high reproduction rate results in a very quick built-up of severe contamination on subsequent host crops. Regular monitoring of *Striga* infestation is therefore an essential component of a control strategy. Farmers in all regions surveyed showed a high interest to experiment with hand-pulling as well as with late weeding (Fig. 1). To suppress *Striga* in the long run the whole package of weeding, including regular monitoring, hand-pulling and late weeding, needs to be employed annually (e.g. a 110 to 130 days sorghum variety would require three to four periods of hand-pulling). Net benefits of this package can be expected only after three to four years. The break even incremental yield of this control package has to be 43 to 55 kg per hectare per annum considering a discount rate of 10% to 20%, respectively. Both herbicides and mineral fertilizer demand cash expense, but with no immediate effect expected. Contact herbicides (e.g. paraquat) applied as a spot treatment will give returns, as will hand-pulling, only after three to four years. In order to produce a pay-off an incremental yield of at least 27 to 37 kg of maize have to be produced per hectare per year. Since these initial cash investments cannot be afforded by most of the farmers this technology has a low adoption potential at the moment. According to a study of the adoption potential of 2,4-D herbicide for *Striga* control in Mali, Debrah and Sanogo (1993) concluded that the maximum farmers are willing to pay represents between 3% and 18% (equalling to about 5.6 to 12.9 US$) of their total annual household incomes. This indicates that expensive technologies with initial investments equivalent to more than 20% of the household's annual income, are not likely to be adopted without access to external sources of financing.

In all regions surveyed it has become obvious that the knowledge about *Striga* biology by farmers as well as by extension workers is very poor. Both farmers and extension workers should be trained in understanding the parasite's biology in order to comprehend and apply the long-term approach for efficient *Striga* management. Therefore, appropriate extension tools as described by Kachelriess et al. (1996) should be made available.

ACKNOWLEDGMENTS

We are grateful to the Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) GmbH which has financed the studies presented within this paper. Special thanks are expressed to the Savanna Agricultural Research Institute (SARI), Tamale, northern Ghana, to the Tanzanian-German IPM-Project , Shinyanga, Tanzania, as well as to the Malawi-German Biocontrol and Post-Harvest Project, Lilongwe, Malawi, for their help conducting the studies.

marketing *Striga* trap crops like cotton or soybeans, farmers have an advantage in their *Striga* control efforts.

Adoption of *Striga* control methods

The interest of farmers in adopting *Striga* control methods in the future did not differ in their main features from one region to another. Control methods which require no financial means like hand-pulling or crop rotation were most attractive. Hand-pulling was thought, depending on the farming system, to be feasible by 54% (Tanzania), 71% (Malawi) to more than 90% (Tamale region of northern Ghana) of the farmers (Fig. 1). Late weeding of *Striga*, which prevents flowering and re-seeding after harvest, is of eminent importance in sorghum as a ratoon crop. Farmers also showed high interest in including this method in their future *Striga* control strategy as it involves no cash outlays.

In contradiction to the high populated areas of northern Ghana, crop rotation especially with trap crops was thought to be a feasible *Striga* control strategy in all other areas investigated. According to long-term field trials of Sprich (1994) in northern Ghana, trap crops such as cotton and soybean reduced the *Striga* seed bank by about 30% annually through inducing ineffective germination whereas conventional cropping of a maize-sorghum mixture increased the *Striga* seed bank by 300% on average. Especially, in the Shinyanga region of Tanzania, cotton production is widespread and therefore possible to include in an effective crop rotation. This is possible only to a limited extent in northern Ghana since cotton is produced on less than 3% and soybean on approximately 0.5% of the total cropped land. In the investigations of Sprich (1994) catch cropping with sorghum and maize was even more effective than trap cropping. The idea of catch cropping is to stimulate germination of *Striga* seeds by host plants but to avoid flowering and seed shed of the parasite through early harvest of sorghum after six to eight weeks. Although sorghum can be used as fodder for animals, in general, only few farmers regarded this method as acceptable, though it could be especially useful in areas where the vegetation season allows a preceeding crop for 6-8 weeks or to restore abandoned fields due to *Striga* infestation.

After having explained the effects of organic manure on *Striga* incidence, especially farmers from the Shinyanga region of Tanzania were interested to apply organic manure more intensively. Particularly in the medium and high populated areas of northern Ghana the use of sufficient quantities of organic manure is unrealistic since the amounts necessary are not available.

Dawoud *et al.* (1996) report that transplanting sorghum from seedbed to the field leads to a decrease of *Striga* infection. In many parts of Africa transplanting of sorghum is used in traditional farming to compensate for the stand loss of sorghum. In the interviews carried out in northern Ghana as well as in Tanzania the method of transplanting sorghum to escape the *Striga* infection was imparted to the farmers which they regarded as feasible on a limited scale, e.g. in highly infested field spots.

The actual low adoption of pesticides in general is reflected in farmers' interest to use herbicides in *Striga* control in the future (Table 2, Fig. 1). Agrochemicals are mainly entering the farming systems through cotton cultivation and since farmers cannot afford them they are not regarded as possible control options. Often, even knapsack sprayers are not available. If nitrogen is used then only at a very low dosage ranging between 15 to 20 kg N/ha. This may even exacerbate the problem as low rates of nitrogen even stimulate *Striga* growth and seed production (Ogborn, 1987). In Bolgatanga, interviewed farmers neither use fertilizer nor any pesticides. Farmers in this area are mainly very poor subsistence farmers, a major constraint for control measures depending on purchased inputs.

region of Tanzania, after insects, *Striga* was classified as the second most important problem and in Malawi *Striga* was known to all farmers as an increasing limiting factor in cereal cropping.

In the past, fallowing fields of more than 10 years was an important farmers' tool to control *Striga*. Due to the rapid demographic growth and the increase in land-use intensity, this technique has to be replaced by other management practices. In northern Ghana for example, related to the population density, the length of the fallow period is very variable. Whereas the fallow period is negligible in the high density system, it is more than 10 years in the low density system (Table 1). In the Tumu area where only about 33% of the agricultural land is cultivated, fallowing is still the most important strategy to control *Striga*. In the medium populated region around 80 % of the cultivated area is cropped. Length of fallow is too short to fight *Striga* effectively. Around Bolgatanga, where population density is highest, fallowing has become impossible. Similarly, fallow periods of 1.6 to 2.7 years practiced in the farming systems investigated in Tanzania and Malawi are too short to reduce the *Striga* seed bank significantly (Table 2). Since *Striga* incidence is closely related to soil fertility (Parker, 1991) and farmers have used *Striga* as a bioindicator showing the depletion of their soils, increasing the soil fertility status using crop residues and organic manure is an important tool in *Striga* management (Ransom and Odhiambo, 1994). In the Bolgatanga and Tamale area, stalks of cereals are collected and stored as a source of fuel while in the vicinity of Tumu crop residues are usually burnt before soil preparation. Organic manure is collected and used systematically in the medium and high population areas, but, due to limited resources, these efforts are mainly restricted to compound fields (i.e. fields nearby the farm households), where especially in the northern Region *Striga* infestation is, compared to bush fields, much lower (Kranz, personal communication). On an average, 55 % and 35 % of the interviewed farmers in the Shinyanga region of Tanzania and in Malawi, respectively, are using organic manure, mainly cow dung from there own cattle herds. Crop residues are either used as fodder or burnt.

In all regions concerned, hand-pulling or weeding for normal weeds is usually performed 1-3 times by farmers in the first four to five weeks after sowing and obviously not with special regard to *Striga*. At this time of the crop growth very few *Striga* plants have already emerged. Therefore, *Striga* growth, flowering and seed production is not prevented. According to Sprich (1994) an additional *Striga* weeding is only performed by 18% of the farmers interviewed in northern Ghana, and only 5% removed the plants to the edge of the fields. In Malawi, 85 % of the farmers left weeded *Striga* plants between the crop rows, and only 15 % removed *Striga* to the edge of the fields, where they burned them. Similar *Striga* weeding practices were found in Tanzania (Reichmann *et al.*, 1995).

Crop rotation is only practiced to a limited extent in the low and medium populated areas of northern Ghana. Usually pseudo-rotations, i.e. rotation of intercrops, prevail. Intercropping is highest in the Tamale area. The diversity of crops cultivated in the low and medium populated area is higher compared to the Bolgatanga area, where only legumes, particularly groundnut and cowpea, and cereals (pearl millet and sorghum) are grown. Compared to northern Ghana and Tanzania, where mixed cropping is also a very common farmers' practice, in Malawi, mainly in the districts of Lilongwe and Kasungu, maize monoculture prevails. With regard to *Striga* control, rotating the cereal and other crops as sole crops in seperate years rather than as mixed crops every year has advantages. However, in general, the proportion of land allocated to cereals is very high in all regions surveyed, meeting the requirements for an optimal *Striga* multiplication. This is largely due to the fact that cereals are grown as the main staple food and in most of the cases intercropped. The introduction of an effective and diversified crop rotation to reduce the *Striga* infestation will be extremely difficult in the Bolgatanga area of Ghana, where aside from cereals only legumes are cultivated. In countries or regions with possibilities for

Introduction

Parasitic weeds of the genus *Striga* (Scrophulariaceae), especially *S. hermonthica* (Del.) Benth. and *S. asiatica* (L.) Kuntze parasitizing cereals as well as *S. gesnerioides* (Willd.) Vatke attacking cowpea, are important biological limiting factors in African agriculture in semi-arid and sub-humid regions. Decades of research have been carried out, but only limited control options can be offered to farmers due to the complexity of the host-parasite interaction (Kroschel *et al.*, 1996a). Benefits of most of the control strategies, e.g. crop rotation, hand-pulling or the use of herbicides (e.g. 2,4-D) will not be immediately apparent. Therefore, an integrated control approach is needed whereby methods have to be applied simultaneously over a period of time. Little is known about the acceptability of control methods by farmers. Moreover, for several reasons (e.g. ethnic groups, population densities, eco-zones) a high variation of farming systems and socio-economic circumstances exist in Africa, for which the adoption of different control methods can be expected. The objective of different case studies carried out in different farming systems of northern Ghana, in the Shinyanga region of Tanzania and in Malawi was to analyze the farming systems in order to identify features which are important with regard to *Striga* management and to evaluate the adoption potential of proposed *Striga* control methods and the constraints involved in implementing them.

Materials and Methods

Since the human population density is the most important determinant for the recent evolution of farming systems in northern Ghana, 69 farmers were interviewed by Runge-Metzger (1993) in three different regions where the population density varied between 8 and more than 120 persons per km², in low (Tumu, Upper West Region), medium (around Tamale, Northern Region) and high (Bolgatanga, Upper East Region) population density systems. *S. hermonthica* occurs in all regions concerned in maize, sorghum and millet, and, according to former studies carried out by Vogt *et al.* (1991), the infection intensity by S. hermonthica is closely related to population density with the highest infestation in the Bolgatanga area. In the Shinyanga Region of Tanzania, 128 male and 12 female (140) farmers were surveyed in the five districts of Bariadi, Maswa, Meatu, Shinyanga and Kahama (Reichmann, 1994). *S. hermonthica* prevail in all regions with the exception of the Kahama district where *S. asiatica* is the most dominant *Striga* species (Reichmann et al., 1995). By way of contrast, the third case study was carried out in Malawi where beside *S. forbesii* Benth., *S. asiatica* represents the most important parasitic weed in cereals (Kroschel *et al.*, 1996b). Here, interviews were conducted with 61 farmers in the three districts of Lilongwe, Kasungu and Mzuzu. In all surveyed areas questionnaires were used. They comprised open and closed questions on the farming system and in particular on the *Striga* situation. The applicability of the questionnaires was pre-tested and adjusted to the specific situation. In addition, several *Striga* control methods were proposed and explained to the farmers in order to evaluate their adoption capacity. Since it was known from previous studies (Shaxson *et al.*, 1993; Sprich, 1994) that farmers knowledge about *Striga* is in general very poor, but an understanding of the parasite's biology implies an important prerequisite to comprehend and apply control methods, the *Striga* biology, i.e. its life cycle was also imparted to them.

Results and Discussion

Characteristics of farming systems with regard to *Striga*

According to farmers' perceptions of the regions surveyed, *Striga* spp. are important constraints in agriculture. In northern Ghana, weeds in general and *S. hermonthica* in particular were mentioned by 98 % of the farmers as the most important biotic problem (Sprich, 1994). In the Shinyanga

Table 1

STRIGA EMERGENCE (11 WEEKS AFTER PLANTING) AND MAIZE YIELD AFTER FOUR AND EIGHT SEASONS OF CONTINUOUS CROPPING WITH TRAP CROPS OR MAIZE UNDER DIFFERING MANAGEMENT SYSTEMS, ALUPE, KENYA

	STRIGA EMERGENCE (PLANTS/M^2)		MAYZE YIELD (KG/HA)	
Treatments	1993 LR[1]	1995 LR	1993 LR	1995 LR
Cotton, no fertilizer	11	23	10	150
Cotton, 50kg N & P	20	5	740	950
Cowpea, no fertilizer	10	12	40	480
Cowpea, 50 kg N & P	25	11	530	750
Fallow	3	14	130	380
Maize, ethylene	1	5	370	1,560
Maize, hand-pulling	6	10	170	1,110
Maize, 50kg N & P, hand-pulling	13	9	2,740	1,880
Maize	5	30	200	580
LSD 0.05	10	13	550	500

1. LR = long rainy season.

Table 2

STRIGA EMERGENCE (11 WEEKS AFTER PLANTING) AND MAIZE YIELD AFTER FIVE AND SEVEN SEASONS OF CONTINUOUS CROPPING WITH TRAP CROPS OR MAIZE UNDER DIFFERING MANAGEMENT SYSTEMS, KIBOS, KENYA

	STRIGA EMERGENCE (PLANTS/M^2)		MAYZE YIELD (KG/HA)	
Treatments	1994 LR[1]	1995 LR	1994 LR[1]	1995 LR
Cotton, no fertilizer	48	13	1,120	1,670
Cotton, 50kg N & P	43	11	2,620	1,350
Cowpea, no fertilizer	65	15	1,040	1,450
Cowpea, 50 kg N & P	80	11	1,870	990
Fallow	39	13	1,230	1,470
Maize, ethylene	8	10	2,990	2,590
Maize, hand-pulling	16	10	1,900	2,410
Maize, 50kg N & P, hand-pulling	14	9	3,300	2,480
Maize	18	11	830	1,650
LSD 0.05	35	NS	1,800	NS

1. LR = long rainy season.

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expected. Even after eight season of continuous cropping, damaging levels of *Striga* remained in the soil. Furthermore, continuous maize coupled with hand-pulling was consistently as or more effective than trap cropping in reducing *Striga* in the following season. The data also indicate that soil fertility can be more limiting than *Striga* as a determinant of maize yield and that low soil fertility can mask the effectiveness that any management practice may have in reducing *Striga* seed numbers in the soil. The confounding effects of soil fertility on *Striga* development and maize growth made interpretation of the results difficult. Certainly with depleted soils like those at Alupe, any *Striga* control program that does not concurrently address the low fertility status of the soil will do little to increase maize productivity.

Adding fertilizer to a trap crop, in the case of cotton at Alupe, increased its effectiveness in reducing *Striga* numbers. This would be expected in a low-fertility soil, as a fertilized crop would have a much more expansive root system. The lack of difference between the cowpea with and without fertilizer, may be due to its N fixing capacity allowing greater growth even in low N conditions.

We did not screen the cultivars of cowpeas and cotton used in this experiment for *Striga* germination stimulant production. Since large differences in stimulant production between cultivars has been found (Berner *et al.*, 1995) it is possible that the poor results we observed from these trap crops were caused by low stimulant production by these particular cultivars. Hand-pulling *Striga* was found to be beneficial in reducing *Striga* numbers and increasing maize yield. However, it should be noted that it is a long slow process, and that even after eight seasons in the case of Alupe, significant and potentially damaging quantities of *Striga* seeds remain in the soil. The data do not indicate that hand-weeding is more effective in a fertilized maize crop than an unfertilized crop.

Ethylene was found to effectively reduce *Striga* numbers. It did not completely eliminate *Striga* at either site even after seven or eight applications (once per season). These data suggest that ethylene is less effective against *S. hermonthica* in this environment than it is against *Striga asiatica* in the USA (Egley *et al.*, 1990). This may be due to a high rate of *Striga* dormancy at this environment (Ransom and Njoroge, 1991). Even though ethylene is a relatively inexpensive pesticide, there is no indication that ethylene would be cost effective, if fertilizer was not applied as part of the package.

The natural weedy fallow provided similar *Striga* control as the unfertilized trap crops. Equal numbers of broad leaf and grassy weeds developed in these fallow plots. The grasses did not support *Striga* parasitism but some of the species may have produced *Striga* germination stimulants. In order for fallow to be more effective, species which have a beneficial effect on soil fertility and induce *Striga* germination should be introduced into the system. Nitrogen fixing trees have been found to meet this criteria (Oswald *et al.*, 1996), and are being tested in a managed fallow system.

The universal and large decline in *Striga* emergence at Kibos between the sixth and eight seasons, regardless of treatment, is significant. Even in the control where *Striga* reproduction was not curtailed, *Striga* pressure declined. This decline was not however reflected in higher maize yields. The high rate of *Striga* seeds mortality at this site is thought to be caused by a soil micro-organisms (Pieterse *et al.*, 1996). Research is needed to identify the organisms involved and develop management strategies to foster their build-up and activity in the soil. Soils with a high *Striga* seed mortality rate should allow continuous maize cropping at economic levels, with little or no other *Striga* control practice needed.

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rains) was hand planted. A spacing of 75 cm by 50 cm (2 plants/hill) was used resulting in a plant population of 53,000 plants/ha. All plots except the weedy fallow were kept free of all weeds except Striga by hand-pulling. For the trap crops, a dual purpose cowpea (variety 'M15') and a commercial cotton variety ('BKA 75') were used. *Striga* counts were taken from the six center rows of each plot after the first emerged *Striga* plant was observed and continued after every two weeks until maize reached physiological maturity. *Striga* counts were then converted to plants/m2. Where hand-pulling of *Striga* was to be done, this started when the first blossom appeared in any of the plots. In most cases two to three hand-pullings was done before the crop matured. All fertilizer was applied at the time of planing in treatments requiring it. The ethylene treatment was applied at 3 weeks after planting maize at a rate of about 15 kg/ha. Maize was harvested from the six center rows and grain yield determined and adjusted to 15% moisture content.

The data were subjected to statistical analysis of variance. Treatment effects were separated using the Least Significant Difference (L.S.D.) method.

Results

The beginning level of *Striga* infestation was high at both sites (data not shown). At Alupe, after four seasons (1993 LR), *Striga* density was the greatest in the treatments which had continuously been cropped to cowpeas or cotton and had been fertilized, or in fertilized maize (Table 1). This was largely due to the fact that the soils at this site had been seriously depleted of its fertility after four seasons of cropping, and only in the plots which had previously been fertilized was there sufficient maize growth to support *Striga*. This is further substantiated by the fact that plots receiving fertilizer were the highest yielding, even while supporting the most *Striga*. Among the various treatments, continuous maize with ethylene resulted in the lowest *Striga* numbers, and continuous maize with fertilizer and hand-pulling of *Striga* resulted in the highest yields.

At Alupe, after eight seasons of cropping, fertilizer was applied uniformly to all treatments to minimize the confounding effects of crop vigor and *Striga* density in the soil on *Striga* parasitism and maize yield. All treatments except unfertilized cotton reduced *Striga* numbers compared to the control (continuous maize with no fertilizer and no hand-weeding *Striga*) (Table 1). Cotton with fertilizer, and maize with ethylene were the most effective treatments in reducing *Striga* numbers. Although there was no doubt some significant carry-over of fertility in the continuously fertilized treatments, there was greater correlation between *Striga* numbers and yield this season. The best yield was obtained with continuous maize with hand-weeding and fertilizer, and continuous maize plus ethylene. Continuous maize with no fertilizer and hand-pulling yielded as well or better than any of the trap crops or the weedy fallow.

At Kibos, there were large difference between treatments after five seasons in both *Striga* numbers and maize yield (Table 2). Treatments with continuous maize generally supported less *Striga* than those with trap crops. Cotton was significantly better than cowpeas when fertilized. The most effective treatment was continuous maize with ethylene. The woil fertility status was much higher at Kibos compared to Alupe and yields were higher in both the fertilized and unfertilized plots. All treatments yielded better than the continuous maize control. The highest yielding treatments were continuous maize with fertilizer and hand-weeding, followed by continuous maize plus ethylene followed by continuous cotton with fertilizer.

After seven seasons, however, there were no significant differences between treatments in both *Striga* density and maize yield (Table 2). Furthermore, there was a large decline in *Striga* emergence in regardless of treatment, including the control where Striga seed production was not curtailed.

Discussion

In general terms, trap cropping was far less effective in reducing *Striga* numbers than was

Introduction

Striga hermonthica (Del.) Benth. is an important pest of maize, the most important cereal in western Kenya. Maize production in limited by heavy infestations (Ivens, 1967). *Striga* is difficult to control especially with existing technology adapted to the small-scale farmers of eastern Africa; *Striga* produces a large number of seeds which survive for up to 20 years in the soil (Khadir, 1983). Furthermore, much of the deleterious effects of *Striga* on the host crop is exerted before emergence from the soil, making hand-weeding, the traditional form of weed control in Africa, ineffective in preventing yield loss. Many farmers have be forced to quit growing maize on their land because of the buildup of *Striga*.

Research in the past has shown that trap crops can effectively reduce *Striga* seed levels in the soil (Cook *et al.*, 1972). Trap crops can be grown as intercrops, relays or in rotation. Parkinson *et al.* (1986) found that soybeans, cotton and bambara nuts were effective trap crops in western Africa. Growing a susceptible crop together with hand-pulling of *Striga* before seed set could also reduce seed numbers in the soil. Carson (1985) reported that most farmers in Gambia control *Striga* by weeding or hand-pulling of *Striga* late in the season when most of the *Striga* have flowered. One season of hand-pulling cannot be successful as *Striga* related damage occurs before *Striga* emerges. Although repeated hand-pulling could be effective, it is tedious and labor intensive especially in heavily infested fields.

The vigor of the crop may have an effect on its ability to stimulate *Striga* germination. Soil fertility is an important determinant of the vigor of the crop. Parker (1984) found that nitrogen can reduce stimulant production by the crop, slow *Striga* development and increase crop tolerance. Mumera and Below (1993), also found a reduction in *Striga* density with the application of nitrogen. Furthermore, Kabambe (1991) observed that nitrogen was more beneficial when applied in the seed bed rather than top-dresses.

Another option available for *Striga* control is the use of germination stimulants. Ethylene gas is the most inexpensive and effective of the stimulants discovered to date and has been used successfully in USA in the *Striga asiatica* (L.) Kuntze eradication program (Eplee, 1975). Its effectiveness against *Striga hermonthica* has not been consistent under western Kenyan conditions (Ransom and Njoroge, 1991).

The objective of this experiment was to evaluate the effectiveness of trap crops, fallow and continuous maize under varying levels of management on the rate of reduction of *Striga* seed banks in heavily infested soils.

Materials and Methods

Field experiments were conducted at the Kenya Agricultural Research Institute's Alupe sub-center near Busia town (orthic FERRALSOLS; partly petro-feric phase; with orthic ACRISOLS) and the National Sugar Research Center-Kibos (vetro-eutic PLANOSOLS). The experiment at Alupe was started during long rains (March-August) of 1991 and at Kibos during the short rains (October-January) of the same year.

The experimental design was a randomized complete block with 3 replications. Treatments are summarized in Table 1. The same treatments were applied to the same plots every season. After four (Alupe) or five (Kibos) seasons of treatment, the plots were split and maize was planted in half of the plot to monitor *Striga* levels. During the final season of the experiments in 1995 long rains, the entire experimental area was planted with maize fertilized with 50 kg/ha of N and P. In the case of the trap crop and fallow treatments, all measurement this season were made on the portion of the plot that had not been grown to maize previously.

Plots consisted of ten rows each 8 metres long. Although plot sizes were large, trenches were dug along the blocks to prevent erosion of soil and land preparation was done by hand. This helped reduce *Striga* seed movement to other plots. Hybrid maize (H512 during long rains and H511 during short

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Future

Research. Emphasis in *Striga* research will be the definition and regional conduct of ISM trials using available technologies. At the Sorghum Projects Meeting held in Bamako, Mali from 9-14 December 1995, the following countries were recommended for participation in this activity: in West and Central Africa (WCA), Burkina Faso, Cameroon, Mali, Niger and Nigeria; in Southern and Eastern Africa (SEA), Botswana, Ethiopia, Kenya, Sudan, Tanzania and Zimbabwe.

Training activities. Three categories are included: 1) Academic: training of students; 2) Group: collaborative (PASCON/ICRISAT/IITA) training courses for NARS technicians engaged in *Striga* research; ICRISAT also participates in training through the GTZ/Univ. of Hohenheim supra-regional project, Ecology and Management of Parasitic Weeds, and; 3) individual: working visits of scientists and technicians.

Extension/technology-transfer activities resulting from technology generated by NARS and/or ICRISAT will be conducted by NARS and facilitated by ICRISAT.

Through assuming a leadership role and intensification of collaboration with its partners, ICRISAT will contribute to effective *Striga* management on and increased and sustained yield of pearl millet and sorghum in the African SAT.

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chemical and biocontrol approaches as they become available. Success in managing *Striga* infestations and reducing damage to the cereal host will depend on consistent and long-term application of ISM technologies. The regional networks, West and Central African Millet Research Network (WCAMRN) and West and Central African Sorghum Research Network (WCASRN), are potentially instrumental in the regional evaluation of ISM techniques.

A secondary research objective is the development of new cultural, chemical and biocontrol components for ISM. Active collaboration with other institutes (e.g., NARS, IARCs and universities) is providing basic information which will lead to new techniques to be incorporated into tomorrow's ISM strategies. The potential of fungal antagonists to control *Striga* under West African conditions is being evaluated (in collaboration with the University of Giessen/GTZ and McGill University/IDRC) and may lead to the development of an effective and environmentally friendly alternative to chemical herbicides for *Striga* control. (e.g., Abbasher *et al.*, 1996; Ciotola *et al.*, 1996). Varietal resistance is an inexpensive and potentially effective method for controlling *Striga*. Using resistant materials identified in the region (Hess and Ejeta, 1992; Obilana *et al.* 1993; Ramaiah, 1991), ICRISAT breeders are working with NARS scientists to develop diversified. *Striga*-resistant populations, parental lines and cultivars. Collaboration with IACR-LARS, Bristol, UK, contributed to the understanding of pre- and post-infection resistance mechanisms in eight SAR lines (Lane *et al.* 1995). A collaborative project with Hohenheim University/GTZ is designed to employ molecular markers to identify genes for qualitative and quantitative resistance to *Striga* in sorghum (Haussmann, *et al.*, 1995). Additional goals are to study the inheritance of resistance in various sorghum lines and develop specific resistance assays.

Although *Striga* incidence and distribution data in Africa have sometimes been collected through national programs and special projects, such information is generally hard to come by. In cooperation with regional institutes (e.g., the Institut du Sahel [INSAH]) and NARS, we are drawing on existing knowledge to accurately describe *Striga* incidence and severity on cereals (e.g., Mbwaga and Obilana, 1994). At the same time information is being obtained on natural enemies to support activities investigating the potential for biocontrol and assess farmers' perceptions of management strategies.

Collaboration and Coordination. PASCON. *Striga* is a regional problem and its management can only be achieved through regional collaboration and coordination. To help achieve this, the Pan-African *Striga* Control Network (PASCON) was formed through a joint FAO/OAU initiative with the objectives: 1) promotion of collaborative *Striga* research and control activities in Africa; 2) strengthening of NARS in *Striga* research and control; 3) facilitation of information dissemination and exchange. Presently, 27 countries, several IARCs (including ICRISAT) and a variety of non-Governmental Organizations (NGOs), interafrican regional organizations and specialized laboratories are active members. Although PASCON workshops provide a useful forum for information exchange on current *Striga* research, funding constraints have hindered the development of a functioning *Striga* research, training and extension network.

IARC Integrated Pest Management (IPM) Initiative. The Consultative Group for International Agricultural Research (CGIAR) recognizes the need to promote IPM using natural and cultural control processes. The major activities of the system-wide IPM Initiative include: 1) establishing a database; 2) information preparation and dissemination; 3) facilitating the development of (8) proposed projects and 4) identification and elaboration of new projects. IITA is coordinating the development with ICRISAT, CIMMYT, and ICARDA, of the Parasitic Flowering Plants Initiative whose objectives include 1) control and containment of parasitic plants at the farm level; 2) improved soil fertility through IPM measures; and 3) increased and sustained crop production.

subsistence farmers (hand-pulling, crop rotation, burning) were investigated in close collaboration with the national program (Institut d'Economie Rurale).

Millet *Striga* research was conducted through the Pearl Millet Improvement Program at ICRISAT's Sahelian Center near to Niamey, Niger. Although *Striga* is a destructive weed, it is important to recognize that multiple factors contribute to grain yield reduction in marginal fields. In a handpulling experiment conducted at two locations in Niger, regular (every 2 wk) *Striga* removal strongly reduced infestation in pearl millet fields from the second year (Fig. 1). A corresponding increase in grain yield was observed at Bengou (Fig 1A), but on less fertile soils at Sadoré, grain yield slowly declined over the five yr of the experiment (Fig. 1B). Thus, management strategies for subsistence farmers should target both *Striga* seed bank reduction and enhanced host productivity. Traditional technologies including crop association, crop rotation and delayed planting were adapted for *Striga* management. Rotation of a nonhost (groundnut) with pearl millet reduced the *Striga* seed bank while improving grain yield (Fig 2). Intercropping with another nonhost, cowpea, was less effective than the rotation (Fig 2). Development of simple laboratory assays permitting selection of nonhosts for ability to stimulate *Striga* seed germination may permit selection of effective nonhosts to employ in rotation or intercropping systems (Alabi *et al.*, 1994). Other strategies including delayed sowing at high plant population (Hess and Williams, 1994) and transplanting from *Striga*-free nursery beds (Berner and Ikie, 1994; D.E. Hess, unpublished data) can contribute to effective *Striga* management in Africa.

Southern and Eastern Africa (SEA). Although *Striga* is present in dry, agriculturally marginal areas of the Southern African Development Community (SADC), it was identified by National Agricultural Research Systems (NARS) scientists as an economically significant production constraint in only three countries (Botswana, Tanzania and Zimbabwe). Together with NARS scientists, the regional SADC/ICRISAT Sorghum and Millet Improvement Program (SMIP) at Matopos works to control *Striga* through breeding of resistant sorghum lines. Of the nine endemic *Striga* species, three (red-flowered *S. asiatica*, *S. forbesii* and *S. hermonthica*) were identified to cause significant damage to sorghum in Zimbabwe, Botswana, and Tanzania (Riches *et al.* 1987; Obilana *et al.* 1988).

Between 1987 and 1992, testing and screening of 490 sorghum germplasm from 10 SADC countries, together with 35 sorghum lines (SAR lines) selected earlier for resistance to *S. asiatica* in India occurred. Using 'hot-spot' fields at Sebele, Botswana; Ukiriguru and Humbolo, Tanzania; and Kwekwe, Zimbabwe; a total of six genotypes were identified as resistant to the three species (Obilana *et al.*, 1991). The genotypes selected were: SAR16, SAR19 and SAR35 for red-flowered *S. asiatica* in Botswana; SAR29 and SPL38A x SAR29 for *S. hermonthica* and red-flowered *S. asiatica* in Tanzania; and SAR19, SAR29 and SAR33 for *S. forbesii* and red-flowered *S. asiatica* in Zimbabwe. Only 2.9% of the sorghum germplasm showed resistance to *S. asiatica* red flowered and *S. forbesii* in Botswana and Zimbabwe. Resistance to all three species was identified in SAR 19 and SAR 29.

Present

Research at ICRISAT has been restructured so that projects, and no longer programs, are the basic unit of operation and management. *Striga* management research is conducted through Integrated *Striga* Management (ISM) subprojects within the global the Pearl Millet Projects and Sorghum Projects. The principle research objective is to facilitate the definition of ISM trials using available technologies in collaboration with national scientists of countries in which *Striga* is a serious problem. Strategies are based on cultural techniques which reduce *Striga* seed number in the soil and stabilize crop yield with supplemental components drawn from the varietal resistance,

Introduction

Witchweed (*Striga* spp.) is endemic to the subtropical regions of the world. The genus belongs to the family Scrophulariaceae and comprises about 36 species, of which approximately 31 occur in Africa (Raynal-Roques, 1987). Most known species are annuals and all are root parasites of flowering plants (Tarr, 1962). Five species attack cultivated cereals: *S. hermonthica* (Del.) Benth, *S. aspera* (Willd.) Benth., and *S. forbesii* Benth. in Africa; *S. asiatica* (L.) Kuntze in both Africa and Asia; and *S. densiflora* Benth. in Asia, particularly India. Only three species are considered to be of widespread economic importance in Africa (Doggett, 1984). Of these, two species cause economic losses to cereals: *S. asiatica* and *S. hermonthica* (Doggett, 1970). *Striga asiatica* occurs mainly in Southern and Central Africa while *S. hermonthica* predominates in Eastern, Central and Western Africa (Doggett, 1970). *Striga hermonthica* attacks both food crops (sorghum, pearl millet, maize, upland rice, sugarcane) and several wild grasses (Tarr, 1962). The parasite produces large numbers of tiny seeds which remain viable in the soil for many years, germinating only when a host root grows in close proximity. Once established in farmers' fields, it is virtually impossible to eradicate.

Detailed information on *Striga* occurrence and yield loss is generally unavailable. In Benin, Cameroon, Gambia, Ghana, Nigeria, and Togo, a mean of 48% (40-77%) of grain fields were estimated to be infested by *Striga* (Sauerborn, 1991). Yield losses averaged 24% (10-31%) but in areas of heavy infestation, losses reached 90-100% in some years. Loss in total grain production averaged 12% (3-26%). Grain production is threatened by *Striga* on 44 million ha in Africa (3.2% of the world's arable land) and loss of revenue from infestation may total 3-7 billion US$ (M'Boob, 1986; Sauerborn, 1991). Favored by low fertility and unreliable precipitation; *Striga* most severely affects subsistence farmers who are least able to afford existing control methods (Boukar *et al.*, 1996).

Striga Research at Icrisat

Past

ICRISAT Asia Center (IAC). *Striga* research at IAC, Patancheru, India, began in 1974 and was active until 1979 under the direction of Dr. K.V. Ramaiah. Breeding material developed has provided promising sources of *Striga* resistance to various African countries. Through screening, varieties including: N 13, 555, IS 4202, IS 7471 and IS 9985 were identified as useful resistance sources. In 1979 ICRISAT's *Striga* research was transferred to Kamboinsé, Burkina Faso, West Africa, and *Striga*-related work at IAC was reduced to a collaborative research project with the Indian Council of Agricultural Research targeting development of *Striga* (*asiatica*) resistant male sterile sorghums.

West and Central Africa (WCA). Emphasis continued on identifying sources of resistance to *Striga* in sorghum. International nurseries conducted in more than a dozen countries, permitted identification of materials with narrow resistance (e.g., IS 9830 and SPV-103) or broad spectrum stable resistance (e.g., IS 6961, IS 8686, Framida and N 13). From 1982, research broadened to include breeding high-yielding resistant sorghums with good food quality and investigation of integrated management systems. Weeding of *Striga*, although effective at reducing infestation, was labor-intensive and frequently did not affect crop yield. Application of 75-100 kg/ha of N both improved sorghum yield and reduced *Striga* emergence. Delayed planting of cereals reduced *Striga* emergence but was accompanied by strong reduction in grain yield.

In 1987 the sorghum *Striga* research effort was moved to the West African Sorghum Improvement Program (WASIP) in Bamako, Mali. Until 1994, work was headed by a weed scientist on a joint CIRAD/ICRISAT appointment. Areas of activity included: *Striga* surveys; developing awareness of the witchweed problem and investigations into *Striga* biology. Methods of control for commercial farmers (N-fertilization, herbicides) and

ranging from 11.80 ^{0} N. latitude to 7.17 ^{0} N. latititude. From each location, the soils were placed into 12 17-cm-diam. pots. Six of these pots from each location were steam pasteurized for 4 hours; the other six were left untreated. One day after pasteurization, all pots were infested with 3,000 germinable *S. hermonthica* seeds. The pots were watered every other day with 150 ml deionized water for 2 weeks. After this period, susceptible maize (cv. 8338-1) seeds were planted in each pot. Maize plants were thinned to 1 per pot after germination. Number of emerged *S. hermonthica* were recorded weekly. At maturity, the maize plants were uprooted and unemerged parasites attached to the roots counted. Maximum number of attached (emerged + unemerged) parasites were statistically analyzed. Results are shown in Table 2. Over all locations there was a highly significant reduction in number of attached parasites of 43% in unpasteurized soils compared to pasteurized soils. These data indicate that natural biotic soil suppressiveness is widespread in Nigeria, including soils from *S. hermonthica* infested areas. Cultural practices that are farmer-acceptable and which enhance natural soil suppressiveness now need to be tested as a complement to rotation.

High Yielding Adapted Cereals with Resistance

Increased and sustained food production is the goal of any integrated pest management program. Increasing human populations in Africa have made high yielding cereal production a necessity. However, to be acceptable to African farmers and to be sustainable in African cropping systems, cereal cultivars must possess good yield potential, good agronomic and storage characteristics, and preferred grain quality even in the absence of pest and disease constraints. Without these attributes, cereal cultivars with resistance to multiple pests and diseases, including *S. hermonthica*, will not be extensively used and will cease to be used, in trade for farmer-preferred cultivars, once the particular pest or disease population has been reduced to tolerable levels. Once these resistant materials drop out of use, pest and disease populations may then again increase. For sustained utility of host plant resistance in IPM, the resistant cultivars must first be farmer-preferable.

A supplement to host plant resistance are host-seed treatments with acethydroxyacid synthase (AHAS) inhibitors. Tests with imazaquin on cowpea showed excellent protection against *S. gesnerioides* and improved plant yields (Berner, *et al.*, 1994a). In maize, phytotoxicity of the AHAS inhibitors on some maize lines has occurred, but excellent *S. hermonthica* protection has been achieved (Berner, *et al.*, 1996). To effectively use these seed treatments on maize in Africa, tolerance to the AHAS inhibitor must first be incorporated into high yielding adapted African maize cultivars. Once AHAS inhibitor tolerance genes are incorporated, the seed treatments essentially function as an externally added gene(s) for *S. hermonthica* resistance.

are counted after 48 hours. For most legume cultivars there is a quadratic response in amount of *S. hermonthica* seed germination as distance the seeds are placed from the root pieces increases. To standardize different germination responses and evaluate relative amount of *S. hermonthica* seed germination induced by each cultivar, the area under the germination curve, reflecting overall germination induction, is calculated with a Genstat^{TM} 5 release 3 computer program for each row of disks in each petri dish. Four petri dishes (replications) are used for each cultivar and statistical analysis is based on individual petri dish means for area under the curve. Only one replication per cultivar is done on any test day. On each test day two petri dishes with no root pieces are included as checks. To one of these dishes 300 µl of distilled water are added to the empty central ring; to the other, 300 µl of an aqueous 10 mg L^{-1} solution of GR24 are added. Areas under the germination curve are calculated as for the other dishes and the germination (area) induced by both distilled water and GR24 are used as covariates in the statistical analysis of cultivars. The assay is simple and inexpensive and is being adopted by national programs for routine testing of promising legume cultivars that are already adapted to the specific locality. A typical range of relative germination efficacy of soybean cultivars from Nigeria is shown in Table 1.

Field validation of the laboratory assay was carried out with separate rotations of 4 soybean cultivars (varying in laboratory evaluated efficacy from extremely efficacious (Soy 1) to no more efficacious than distilled water (Soy 4)), and a cowpea cultivar (ranked as very efficacious in the laboratory) grown prior to planting a local *S. hermonthica* susceptible sorghum cultivar. A sorghum-sorghum rotation was used as a check. The field in which the evaluation was conducted was uniformly infested with *S. hermonthica* seeds at the end of the growing season that preceded the start of rotation study. Each legume cultivar or sorghum was planted in plots consisting of 8 10-m-long rows. Four replications were used. In the season following cultivation of the soybean and cowpea cultivars, the local sorghum cultivar used in the sorghum plots was planted in the respective former legume or sorghum plots. Sorghum grain yield and maximum numbers of emerged *S. hermonthica* per sorghum plot were recorded. Results of this one season rotation are shown in Figure 2. There was a rank-order correlation between laboratory efficacy and emerged *S. hermonthica* of $r = -1.00$ ($P \leq 0.01$). Between laboratory efficacy and sorghum yield the rank-order correlation was $r = 1.00$ ($P \leq 0.01$).

Thus, the laboratory assay was shown to be an accurate predictor of field efficacy of selected legume cultivars in reducing *S. hermonthica* parasitism and increasing sorghum grain yield. The results show the benefit in being able to make an educated specific recommendation for use of effective legume cultivars and the danger in making generic recommendations for crop rotations. A generic recommendation to plant soybeans in rotation with sorghum for *S. hermonthica* control could have disastrous results if the only cultivar available was Soy 4 (Fig. 2). Most significantly, the benefits of an effective legume rotation were demonstrable within only one rotation cycle. Sorghum grain yield increased over 30 times with the Soy 1-sorghum rotation as compared to the sorghum-sorghum rotation and over 6 times in comparison with the Soy 4-sorghum rotation. Maximum numbers of emerged *S. hermonthica* in the Soy 1-sorghum rotation were half that of the Soy 4-sorghum rotation. The substantial and relatively rapid effectiveness of legume cultivar rotations, and the side benefits of improved soil fertility, have also been demonstrated with cowpea (Carsky and Berner, 1995) and *Aeschynomene histrix* (Weber, et al., 1995). Farmer demand in Nigeria for not only soybean but also forage legume seeds has now skyrocketed, and these rotations are the focus of our integrated control program.

Biological Control

To ascertain the extent and degree of natural soil suppressiveness in Nigeria, soils were collected from farmers' fields in 11 locations in Nigeria

VI.15

PROPOSED INTEGRATED CONTROL PROGRAM FOR *Striga hermonthica* IN AFRICA

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ABSTRACT

Control of *Striga hermonthica* under African farming conditions is a complex problem. Prior to recent increases in human population pressure, *S. hermonthica* was controlled in African farming systems by prolonged crop rotations with bush fallow. Because of increasing need for food, these fallow rotations are no longer extensively used, and *S. hermonthica* has become a severe problem in cereal production. What is proposed herein is an integrated program of control that replaces traditional bush fallow rotation, provides food, and improves the agricultural system as a whole.

Key words: alternative agriculture, sustainable agriculture, crop rotation.

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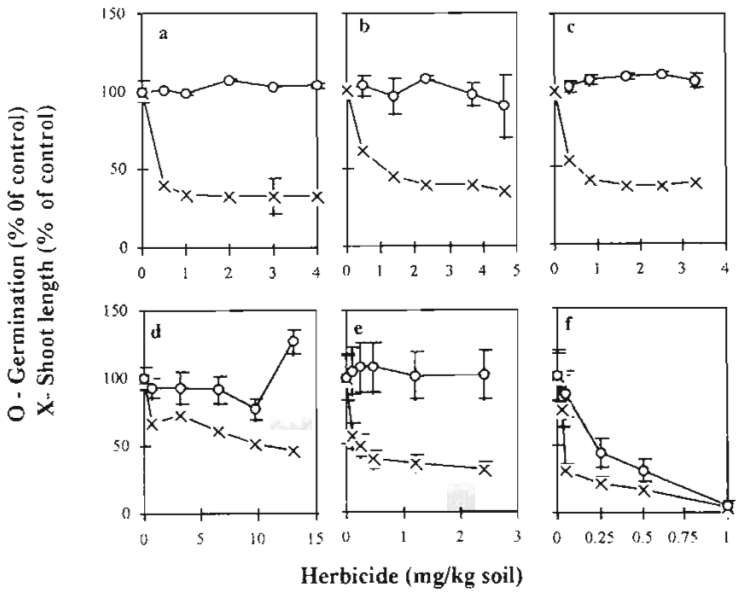

Figure 1

Effect of soil-incorporated herbicides on seed germination (o) and shoot elongation (x) of field dodder. (a) pronamide, (b) pendimethalin, (c) trifluralin, (d) prodiamine, (e) thiazopyr, and (f) isoxaben.

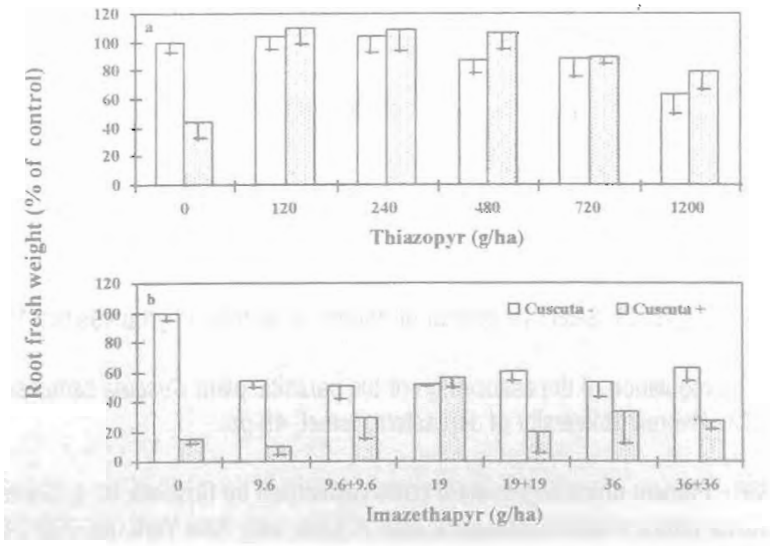

Figure 2

Effect of postemergence application of thiazopyr (a) and imazethapyr (b) on root fresh weight of carrots inoculated and non-inoculated with dodder.

bury freshly produced seeds, as a tool to facilitate the decline of the dodder seed-bank in soil.

Surviving seeds which were recovered from the various soil depths, were tested for vitality. Exhumed seeds which germinated before or after scarification were considered vital. Overall, in spite of the observed fluctuation, the number of vital seeds is declining with time (data not shown). The depth of burial had little effect on vitality of dodder seeds in soil. At the 67th week from burial, the combined germination rate is about half of that measured at the beginning of the experiment. Although this long term experiment has not yet been finished, the partial results available so far, clearly indicate that field dodder can maintain a considerable seed-bank for at least several years.

Response of field dodder to herbicides

The aim of this series of experiments was to screen and select herbicides that inhibit dodder seed germination and shoot elongation, before they parasitise their host plant. At all rates tested, the following herbicides did not inhibit dodder germination nor affected shoot elongation: rimsulfuron, imazethapyr, AC 263,222, imazaquin, atachlor, vernolate, metribuzin, and ethofumesate. Flurochloridone, a known inhibitor of carotenoids biosynthesis has shown some activity but required relatively high rates to inhibit shoot elongation.

Pronamide, at all concentrations examined, did not affect dodder germination, whereas shoot elongation was dramatically inhibited even at a very low concentration of 0.5 mg/kg soil (Fig. 1a). In spite of the fact that they belong to another chemical group, similar results and symptoms were observed with the dinitroaniline herbicides such as pendimethalin (Fig. 1b), trifluralin (Fig. 1c). Prodiamine, on the other hand, was less effective than the other dinitroanilines in reducing shoot length, and required much higher concentration to achieve 50% inhibition (Fig. 1d). Thiazopyr, although not a dinitroaniline, behaves in a similar manner to this group of herbicides and effectively inhibited shoot elongation without any effect on seed germination (Fig. 1e). Isoxaben was the most effective herbicide, which strongly inhibited both seed germination and shoot elongation (Fig. 1f), but its selectivity to carrots is marginal (Nir and Rubin, unpublished). Herbicides affecting cell division and development might be good candidate for arresting the growth and vegetative proliferation of dodder before, or soon after its emergence and attachment to the host plant. One should remember however, that within three days after emergence, dodder is disconnected from the soil. Thus, it probably would be better to use herbicides that are able to be taken up by the host roots, move via its vascular system (xylem or the phloem), reach the parasite with the assimilates or water and injure it.

It was found that both the effect of the parasite and of the tested herbicide on the crop are best reflected by the fresh weight of carrot roots. Thiazopyr, a mitosis inhibitor of the pyridine group, exhibited high selectivity to carrots. Thiazopyr applied post-emergence at 120 and 240 g/ha, showed excellent control of dodder (data not shown) without damaging the carrots (Fig 2a). Thiazopyr applied at a rate of 480 g/ha was injurious to non-inoculated carrots and reduced their root fresh weight. However, when dodder-inoculated plants were treated with the same amount of thiazopyr, no damage was observed. As expected, thiazopyr at 1200 g/ha caused a higher level of injury to the non-parasitised carrots, but was significantly less injurious to those inoculated with dodder. These data indicate that at least part of the absorbed herbicide has been removed from the host plants and accumulated in the parasite, so that a lower amount of the herbicide remained in the host. Similar results were reported for pendimethalin in dodder associated with bean plants (Liu and Fer, 1990). Since thiazopyr is not a phloem-mobile herbicide, further translocation studies are needed to support this hypothesis, and better understand the mechanism involved.

Imazethapyr a known inhibitor of branched amino acids biosynthesis, has shown low selectivity to

Field dodder control

Experiments were performed under controlled conditions (25C), using commercially available herbicides at various concentration, expressed as active ingredient. Herbicides were thoroughly mixed in Rehovot soil, the soil brought to 50% field capacity and put in petri dishes (90x15mm). Twenty dodder seeds were planted in one row, and covered with a layer (2mm) of treated soil and the lid. The petri dishes (5 replicates/treatment) were held in the dark, at 60 angle, to allow shoot growth on the soil surface. Six days after planting, emerging dodder seedlings were counted and their length measured. Herbicides tested were: pendimethalin, trifluralin, prodiamine, pronamide, thiazopyr, isoxaben, rimsulfuron, imazethapyr, AC-263,222, imazaquin, flurochloridone, alachlor, vernolate, metribuzin, and ethofumesate. Carrot plants were grown in pots to the 3 leaf stage, then 10 acid-scarified dodder seeds were planted in each pot. Herbicides were applied (5 replicates) post-emergence at various rates starting when dodder haustoria were connected to the host. Host and parasite injury were visually rated periodically. Five to six weeks after treatment, carrot plants were harvested, shoot (with the attached dodder) and roots separated and fresh weight determined.

Results and Discussion

Field dodder germination and emergence

Germination started at 10°C, but very slowly, such that more then a month was needed before first germinating seed was observed (data not shown). Most seeds (80%) germinated within 2 to 4 days at temperatures between 20°C and 35°C, whereas 11 days were needed at 15°C. At 40°C, germination was below 20%. No differences were observed in dodder germination rate between seeds that were in dark or in continuous light. Field dodder emergence is largely influenced by the depth of sowing with maximum emergence occurring within 2 days on the soil surface. At deeper sowing depths, the emergence was delayed and speed of emergence decreased. No seedlings emerged from depths below 10 cm. When similar experiments were conducted in a silty-clay soil (Givat Brener, 41% clay) emergence was ca 75% at 0.5 and 2 cm depths, but declined to 25% from 5 cm. In Alumim soil (clay-loamy loess, 28% clay) emergence was very poor at all depths tested (20% at 0.5cm, 5% at 2 cm, and no emergence observed from 5 cm), perhaps due to the hard crust that rapidly develops on loess soils.

Our results are generally in agreement with information reported by Hutchison and Ashton (1980) in California. The wide range of potential host plants and the adaptation of field dodder seeds to germination in a wide range of temperatures, allow the parasite to germinate and develop in places like Israel almost the whole year round. Moreover, the seedlings capacity to emerge from a deep layer of the cultivated soil (0 to 8 cm), further broaden the ecological niche where it can parasite, which may explain why field dodder is such an extremely troublesome weed.

Seed burial

In the three burial depths examined (5, 10 and 20 cm), the number of seeds remaining intact gradually declined with time. During the first 10 weeks (starting December 1993), a sharp decline by 20 to 25% in the number of the remaining seeds was observed, followed by much slower rate of seed decay. During the winter and spring of 1995 however, the number of remaining seeds declined again thus, on the 67th week from burial, the number of surviving seeds is 60, 80 and 40% of the initial number at 5, 10 and 20 cm, respectively (data not presented). The differences between the shallow burial depths (5 and 10 cm), and the 20 cm depth in terms of the number of recovered seeds, increased with time. It seems that the missing seeds either germinated inside the nylon mesh or decayed in such a way that we were not able to identify them. These data may indicate the potential of using soil cultivation, such as ploughing that can

VI.14

On the Biology and Selective Control of Field Dodder (*Cuscuta campestris*)

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ABSTRACT

Field dodder (*Cuscuta campestris*), a non-specific parasitic weed, attacks a wide range of host species world-wide. The parasite coils around the host, penetrates its tissue via haustoria, and withdraws assimilates, nutrients and water, hence reducing crop quality and yield. The effect of edaphic and environmental conditions on dodder germination and development as well as its response to herbicides were studied in laboratory and field experiments. In petri dishes, no seed germination was observed below 10C or above 40C, with maximum at 15C to 35C. In a sandy soil (Rehovot), maximum emergence occurred from the soil surface, with most seeds emerging within 3 days. Deeper sowing and sowing in heavier soils resulted in a reduced and delayed emergence, with no emergence below 10 cm. The viability of seeds buried at various soil depths in the field, gradually declined with time. Experiments have shown that soil-incorporated herbicides, known as cell division and development inhibitors (dinitroanilines, pronamide, thiazopyr and isoxaben), inhibited dodder germination and arrested its vegetative growth before or soon after attachment to the carrot plant. In post-attachment treatments we examined the hypothesis that dodder employs a strong sink ('super sink'), hence phloem-mobile, non-selective herbicide, applied on the host will rapidly be withdrawn by, and accumulate in, the parasite. Preliminary results have shown that carrots infested with dodder treated with certain rates of thiazopyr or imazethapyr were less injured as compared to uninfested plants. These data indicate the potential of using low rates of non-selective herbicides for selective control of parasitic weeds.

Additional key words: seed burial, herbicide, sink, phloem mobility.

Table 1

NATIVE AND WEEDY SPECIES OF *CUSCUTA*, *OROBANCHE* AND *STRIGA* REPORTED TO OCCUR IN AUSTRALIA TO 1995

Species	Naumes used in error or previously	State[a]	Status	References
Striga				
curviflora Benth.	*multiflora* Benth.	NT,Q,W	native, not weedy	Barker 1990, 1992a, 1992b
gesnerioides (Willd.) Vatke ex Engl.	*orobanchoides* (R.Br.) Benth.		record erroneous	Holm *et al.* 1979
asiatica (L.) Kuntze	*lutea* Lour		record erroneous	Bentham 1869; Hnatiuj, 1990; Lazarides & Hince, 1993; Yamazaki, 1985; Guymer, pers. comm. 1995
hirsuta Benth.		Q	recorded, not naturalised, extinct	Bentham, 1869
parviflora Benth.		NT,Q,W	native, insignificant weed	Kleinschmidt & Johnson, 1977; Stanley & Ross, 1986
squamigera W.R.Barker		NT,W	native, not weedy	Barker 1990, 1992a, 1992b
Orobanche				
aegyptiaca Pers.			record erroneous	Carter & Cooke 1994
cernua Loefl. var *australiana* (F. Muell. ex Tate) J. Black ex G. Beck		N,S,T,V	native, rare	Barker 1986, 1992; Carter & Cooke 1994
var *cernua*			record erroneous	Carter & Cooke 1994
crenata Forsk.			record erroneous	Carter & Cooke 1994
minor Sm.	*australiana* auctt.	N,S,T,V,W	minor weed, widespread	Carter & Cooke 1994
ramosa L.	*mutelii* F.W.Schultz	S	recorded, eradication in progress	Barker 1986; Carter & Cooke 1994
Cuscuta				
australis R. Br.		N,Q,V	presumed native, rare	Johnson 1992; Willis 1972
epilinum Weike		V	recorded, not naturalised	Willis 1972
epithymum (L.) Murr.	*europaea* L.	N,Q,V,T,S,W	recorded, either not naturalised or rare	Johnson, 1992; Parsons 1973; Willis 1972
europaea L.		V	recorded, either not naturalised or rare	Willis 1972
indecora Choisy		V	recorded, rare	pers. obs. Cooke 1995
pentagona Engelm.	*campestris* Yuncker *arvensis* Beyrich	N,Q,S,V,W	widely scattered, minor weed,	Hom *et al* 1979; Johnson 1992; Parsons & Cuthbertson 1992
planiflora Ten.		S,V,W	rare, eradication programs in progress	Johnson 1992; Willis 1972
racemosa Mart.		V	recorded, not naturalised	Willis 1972
suaveolens Ser.		N,S,V	rare, eradication programs in progress	Johnson 1992
tasmanica Engelm.		N,S,T,V,W	native, rare	Johnson, 1992; Willis 1972
victoriana Yuncker	*tatei* Yuncker	N,NT,Q,S	native, uncommon	Johnson 1992

a. N = New South Wales, NT = Northern Territory, Q = Queensland, S = South Australia, T = Tasmania, V = Victoria, W = Western Australia.

VI.13

OCCURRENCE AND CONTROL OF *Striga, Orobanche* AND *Cuscuta* IN AUSTRALIA

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ABSTRACT

Indigenous Australian *Striga*, *Orobanche* and *Cuscuta* species are not weeds of agriculture. Many authors perpetuate confusion between the native Australian species: *Striga curviflora*, *S. squamigera*, *S. parviflora*; *Orobanche cernua* var. *australiana*; *Cuscuta australis*, *C. victoriana* and *C. tasmanica* with weedy relatives. *Cuscuta pentagona* is the most important parasitic weed in Australia. *C. epithymum*, *C. indecora*, *C. planifora* and *C. suaveolens* are rare. Control authorities are containing or eradicating all introduced *Cuscuta*. *O. minor* is scattered widely in south-west and south-east Australia, but is an insignificant weed. *O. ramosa* is subject to an eradication program. Reports of *Striga asiatica*, *S. hermonthica*, *S. aspera*, *O. cernua* var. *cernua* and *O. cernua* var. *cumana* are erroneous. They do not occur in Australia.

Additional key words: quarantine, range, spread, impact.

Table 1

STRIGA SEEDS RECOVERED FROM THE SOIL OF TWO POT EXPERIMENTS USING SEVEN TREE SPECIES TO INDUCE *STRIGA* SEED GERMINATION, AS A PERCENT OF THE BARE SOIL CONTROL

Tree Species & Controls	*S. hermonthica*	*S. asiatica*	Mean
Soil (control)	100 a	100 a	100
Gliridicia sepium	79 ab	91 ab	85
Leucaena leucocephela	73 bc	86 abc	80
Croton megalocarpus	63 bcd	84 abc	74
Markhamia lutea	58 bcd	54 d	56
Maize (H512)	56 cd	45 d	51
Sorghum (Serena)	51 d	58 cd	55
Sesbania sesban	50 d	66 bcd	58
Calliandra calothyrsus	48 d	107 a	78
Leucaena diversifolia	47 d	68 bcd	58
Coefficient of variation	27	27	

For each species seperately, means followed by the same letter within a column are not significantly different at the 0.05 level according to Duncan's multiple range test.

Table 2

GERMINATION OF *STRIGA* SEED BY VARIOUS SPECIES AS A PERCENT OF THAT INDUCED BY SORGHUM IN TWO ROOT CHAMBER EXPERIMENTS

Species tested	*S. hermonthica*	*S. asiatica*
Sorghum (control)	100 a*	100 a
Leucaena leucocephela	46 b	–
Leucaena diversifolia	43 b	100 a
Sesbania sesban	20 c	–
Markhamia lutea	15 c	–
Calliandra calothyrsus	15 c	22 b
Gliridicia sepium	–	17 b
Coefficient of variation	25	22

** For analysis, percentage data were transformed using arcsin.*
For each species seperately, means followed by the same letter within a column are not significantly different at the 0.05 level according to Duncan's multiple range test.

study the response of *Striga* on a well developed root system. To use potted plants for screening purposes, the work load for seed extraction and seed counting has to be diminished, and the reason for the consistently low amounts of *Striga* seeds recovered from the soil should be identified.

Among wild plant and tree species there is potential for reducing *Striga* seed banks in the soil. Laboratory screening methods to identify those plants needs further development to give reproducible results. For the final verification of these findings, field trials are essential.

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(hybrid 'H512') were sown as controls and also pots with only soil and *Striga* seeds were used. After three months, *Striga* seeds were extracted from the soil and counted using procedures described by Ndungu *et al.* (1995). Striga seed counts were analysed with an Analysis of Variance and then presented as percentage of counts from the bare soil control (Table 1).

Laboratory experiments

Seeds of the same set of tree species were sent to Germany to be evaluated in a laboratory experiment using root chambers according to the method described by Linke and Vogt (1987) and modified by Vogt (1993). The design was a CRD with three replications. Each root chamber contained one germinated tree seed and *S. hermonthica* or *S. asiatica* seeds. Sorghum (variety 'Mankaraga' from Ghana) was used as a control. *Striga* seeds were sown on the filter paper using a template to apply the same amount of seeds in the same pattern to all chambers. An area of 7.5 mm on either side of the growing roots was marked on the plexiglas of the chambers with a felt-tip pen and the germinated *Striga* seeds within this area were counted at weekly intervals. After five weeks the experiment was terminated. Data were set as percentage of seeds germinated by the control. The percentage data were transformed to arcsin and then analyzed using an Analysis of Variance and means separated using Duncan's multiple range test.

Results and Discussion

Seeds of all tree species except *Grevillea robusta* germinated well and developed into viable plants for the pot experiments in Kenya. After 3 months, less than 20% and 40% of the initial *Striga* seeds were recovered from the elutriation system for *S. hermonthica* and *S. asiatica*, respectively. This was largely thought to be due to natural attrition and not loss in the extraction process (Ndungu, *et.al*, 1995). Only *Gliricidia sepium* failed to reduce *Striga* seed numbers compared to the control, for *S. hermonthica*, and *G. sepium*. *Leucaena leucocephala, Croton megalocarpus* and *Calliandra callothyrsus* for *S. asiatica* (Table 1).

In the laboratory experiments the growth of the tree seedlings was a major problem for the root chamber trial. Several of the trees failed to establish and the slow growth made it difficult to compare the tree species with the cereal check when germinated at the same time. All trees induced some germination, though the rate of germination was very much reduced compared to the sorghum, due to the fact that the germination of the parasite depended on the extend of the hosts´ root system as well as the activity of the exudate. *Markhamia lutea, Calliandra callothyrsus* and *Sesbania sesban*, particularly, induced little germination of *Striga hermonthica*. *Leucaena diversifolia* was as effective as the susceptible crop sorghum in germinating *Striga asiatica* (Table 2).

The two series of experiments showed that some leguminous tree species have the potential to reduce *Striga* seed banks in the soil and could be used as a component in controlling this weed. Out of eight species tested, three (*Markhamia lutea, Sesbania sesban, Leucaena diversifolia*) were consistent in germinating *Striga* across all experiment and their effectiveness should be further verified in the field. The use of *Sesbania sesban* in a managed fallow system directed toward controlling *Striga* appears especially encouraging, as this species can induce high levels of suicidal germination, it can be established by seed, grows quickly relative to other species, fixes nitrogen, and can be easily removed from the field at the end of the fallow (ICRAF, 1994). Other native trees and plants may have similar or better potential for *Striga* control, and additional screening should be carried out.

However, the methods used for this screening need further improvement. For the root chamber technique it is essential to keep the germinated tree and plant seeds alive for a longer period of time, to

Introduction

Within Kenya, *Striga hermonthica* (Del.) Benth infests approximately 158,000 ha in the Lake Victoria basin where farmers have identified it as the most important constraint to maize production (Hassan *et al.*, 1995). *Striga asiatica* (L.) Kuntze is also widely distributed in the coastal region. In these densely populated areas, agriculture is intensive and fallow periods reduced. This causes a steady decline in soil fertility and soil organic matter, which in turn favors *Striga* infestation (Vogt *et al.*, 1991).

A number of annual crop species cause suicidal germination of *Striga* seeds and have been recommended as "trap crops". These may be grown either as intercrops or in rotation with *Striga*-susceptible crops. Mulitipurpose shrubs and trees in agroforestry systems may also be grown simultaneously or in sequence with annual crops. Trees can provide many products and services, such as fuelwood, building materials, fodder, fruits, fibres, mulch, soil conservation, and shade. There has been widespread adoption of boundary plantings, parkland systems, agroforests, and farm woodlots (Bradley, 1991; Hoekstra, 1988; Warner, 1993). For example, on the eastern slopes of Mount Kenya farmers plant *Grevillea robusta* A. Cunn. ex R. Br. (silver oak) to delineate their farm boundaries and as a source of fuelwood and timber. Farmers in Kenya often leave fruit trees and other multipurpose trees, such as *Markhamia lutea* (Benth.) Schumann, *Grevillea robusta* and *Sesbania sesban* (L.) Merr., to grow at wide spacing (>20 m) in crop land - a form of parkland system.

Several other systems have high potential for widespread adoption in Kenya. These include contour hedges for both fodder production and biomass transfer, and improved fallows for soil fertility improvement. Contour hedges are planted to prevent erosion and form biological terraces. If an appropriate species is used, such as *Calliandra calothyrsus* Meissn, *Gliricidia sepium* (Jacq.) Kunth ex Walp., *Leucaena leucocephala* (Lam.) de Wit, or *Leucaena diversifolia* (Schldl.) Benth, prunings may also provide a good source of fodder protein to supplement fodder grasses, like napier grass (*Pennisetum purpureum* Schum.), which may be grown on contour bunds together with the hedges. Alternatively, the biomass can be transferred to the fields as a source of mulch or green manure. Improved tree fallows occupy land that is not cropped for a few months or for two to three years, to accumulate biomass and nutrients as well as to smother weeds. A promising system has been developed in western Kenya whereby *Sesbania sesban* is established along with maize at the start of the first rains. After the maize harvest, the *Sesbania* grows rapidly during the short rains. It may be harvested for fuelwood at the start of the next first rains, when the leaf material is incorporated as a green manure (ICRAF, 1994).

The objectives of these investigations were to screen a variety of fast growing multi-purpose tree species for their ability to reduce the *Striga* seed bank in the soil and to assess the suitability and limits of the techniques used to accomplish this aim.

Materials and Methods

In 1994 and 1995 laboratory and pot experiments were conducted to evaluate the ability of leguminous tree species to germinate seeds of *S. hermonthica* and *S. asiatica* at the National Sugar Research Center, in Kibos, Kenya and the Institute of Plant Production of the Tropics and Subtropics of the University of Hohenheim, Germany.

Pot experiments

A Completely Randomized Design (CRD) with five replications was used to screen seven tree species (Table 1) in two series of pot experiments. In each pot containing 2 kg air-dried soil mixed with approximately 500 seeds of either *S. hermonthica* (series 1) or *S. asiatica* (series 2), one germinated tree seed was placed and then left to grow for three months. Sorghum (variety 'Serena') and maize

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Table 3

NUMBER OF *STRIGA HERMONTHICA* SHOOTS DEVELOPED WITHIN FOUR WEEKS ON SORGHUM ROOTS AS INFLUENCED BY TRANSPLANTING AGE OF SORGHUM[a] (ROOT CHAMBER EXPERIMENT)

Sorghum age at transplanting (days)	Number of *Striga* seeds/along the host roots	Number of shoots along the host roots	Shoots %
0 (control)[a]	215	6.2	2.9 a[b]
7	127	2.7	2.2 b
14	153	2.8	1.8 bc
21	141	2.2	1.6 bc
28	127	1.6	1.3 dc

a. Control treatment consisted of directly sown sorghum at the time of transplanting.

b. significance at $P \leq 0.05$ after square root $(y + 0.5)$ transformation is indicated by various lower case letters.

Table 4

EFFECT OF SORGHUM SEEDLING AGE AT TRANSPLANTING ON *STRIGA HERMONTHICA* AND SORGHUM DRY WEIGHT 12 WEEKS AFTER TRANSPLANTING OF SORGHUM (POT EXPERIMENT)

Sorghum age at transplanting (days)	SHOOT DRY WEIGHT	
	Striga g/pot	Sorghum g/plant
Control 1[a]	5.8 a	8.6 a
Control 2[b]	2.4 b	8.2 a
7	1.8 b	9.4 ab
14	2.0 b	10.4 b
21	2.4 b	12.2 c
28	1.5 b	12.7 c

a. Sorghum sown to Striga-infested pots 28 days prior to the date of sorghum transplanting.

b. Sorghum sown to Striga-infested pots at the date of sorghum transplanting.

Significance at $P \leq 0.05$ is indicated by various lower case letters.

Table 1

EFFECT OF TRANSPLANTING SORGHUM SEEDLINGS ON *STRIGA HERMONTHICA* SEED GERMINATION (ROOT CHAMBER EXPERIMENT)

Sorghum age at transplanting (days)	WEEKS AFTER TRANSPLANTING OF SORGHUM			
	1	2	3	4
	Striga germination %			
0 (control)[a]	36.4 a	46.3 a	48.2 a	48.2 a
7	34.5 ab	38.6 ab	41.7 ab	41.7 ab
14	26.3 ab	31.2 b	32.8 bc	32.9 bc
21	25.9 ab	29.6 b	30.4 bc	30.5 bc
28	16.8 b	26.1 b	27.8 c	27.9 c

** Control treatment consisted of directly sown sorghum to the root chambers at the time of transplanting significante at P ≤ 0.05 is indicated by various lower case letters.*

Table 2

EFFECT OF TRANSPLANTING SORGHUM SEEDLINGS ON THE ATTACHMENT OF *STRIGA HERMONTHICA* (ROOT CHAMBER EXPERIMENT)

Sorghum age at transplanting (days)	WEEKS AFTER TRANSPLANTING OF SORGHUM			
	1	2	3	4
	Striga attachment (%)			
0 (control)[a]	2.0 a	7.4 a	13.6 a	14.4 a
7	2.0 a	5.0 ab	9.3 b	9.3 b
14	1.7 a	3.0 bc	4.0 c	4.2 c
21	1.2 a	2.8 bc	4.2 c	4.6 c
28	0.0 a	0.9 c	4.2 c	4.4 c

** Control treatment consisted of directly sown sorghum to the root chambers at the time of transplanting significante at P ≤ 0.05 is indicated by various lower case letters.*

Effect of transplanting on the development of *Striga hermonthica* in pot trials

In the pot experiment, although transplanting of sorghum significantly improved the crop yield, no consistent effect on *Striga* population and the parasite dry weight compared to the directly sown crop at the time of transplanting was found at harvest. However, directly sown sorghum four weeks before transplanting resulted in a significantly higher parasite shoot dry weight compared to the transplanted treatments (Table 4). The inconsistency in the effect of sorghum transplanting regarding the significant reduction in the number of attached parasite plants in the root chamber experiments and no significant effect of the method on the number of emerged parasites in the pot experiments might be attributed to the fact that under the condition of low *Striga* attack most of the parasite seedlings could emerge while under the conditions of high parasite attack the host plant could support only a low number of attached seedlings to emerge. It is therefore possible to have a similar population of *Striga* plants above ground, although the total number of parasitism may be different. These conditions were also reported using other control methods that reduce the parasite attack such as hand pulling (Doggett, 1965) and herbicide application (Robinson and Dowler, 1961).

Sorghum performance

Under the stress of *Striga* attack, transplanting of sorghum was found to improve host growth compared to directly sown crop (Table 4). Transplanting 28 days old sorghum plants resulted in 12.7 g/plant of the crop shoot dry weight compared to 8.2-8.6 g/plant when directly sown. Transplanted sorghum probably has benefited from escaping early *Striga* attack. Cechin and Press (1994) suggested that the age at which a sorghum plant becomes infected with *S. hermonthica* may be an important determinant of the extent to which the parasite influences crop productivity. They observed a severe reduction on the photosynthesis and height of infected sorghum when inoculated with *S. hermonthica* at 3 days as opposed to 19 days post host germination. Under field conditions, transplanting of sorghum could integrate other control methods such as planting date without reduction of the growing season. Sowing of sorghum after the advancement of the rainy season was found to reduce *Striga* infection (Bebawi, 1987; Parker and Riches, 1993). However, the benefits of crop yield resulting from reduced *Striga* attack will almost certainly be masked by the tendency to reduce yield from delayed planting (Parker and Riches, 1993). Sorghum transplanting will solve this constraint. The crop will be planted early in the season in *Striga*-free nurseries and then will be transplanted later in the season to infested fields. At this time the field conditions will not be favorable for high *Striga* infection due to the advancement of the rainy season.

Transplanting of sorghum could also contribute to the reduction of the risk of crop failure due to the fluctuation of rainfall especially in the northern limits of the semi arid tropics. Since sorghum seedlings could be raised in other places with available water either by irrigation or early rains then transplanted to areas of relatively low rainfall.

The method is simple and requires low skill for its implementation in such a way that it can be done by the subsistence farmer and his family. Sorghum transplanting could also be extended to large production farms such as mechanized rainfed schemes in the Sudan (farm size 420-630 ha.), if the positive results of mechanization of transplanting maize and sugar sorghum developed by Scheffer (1985; 1988) could be adapted to sorghum production. The cost of transplanting could be compensated by the increased crop productivity due to the reduction of *Striga* induced crop losses. Moreover, with sorghum transplanting the cost of many farm operations, such as sowing, resowing, thinning and weeding can be reduced. However, the contribution of the method to more economic production, in addition to its contribution

along the host root. The experiment was terminated four weeks after transplanting of sorghum to the root chambers. Four replicates were used for each treatment which were arranged in completely randomized design. The experiment was carried out twice.

Transplanting of sorghum and its effect on *Striga hermonthica* incidence and sorghum yield

The experiments were carried out in a glasshouse at 35-30/25-20C and 12/12 hours day/night conditions. Plastic pots (18x18x18 cm) filled with 2:1 (v/v) loam : sand were sown with 15 sorghum seeds in weekly intervals for four weeks and thinned a week after sowing to 10 plants/pot. For transplanting sorghum seedlings were extracted with the help of a small stick, while extra soil on the roots was removed carefully by shaking. Two sorghum seedlings per pot were then transferred to pots of the same size which contained 100 mg of preconditioned *S. hermonthica* seeds (collected from Ghana, 1991) in the upper 10 cm. Treatments were direct sown sorghum either four weeks before transplanting or at transplanting date and sorghum that was transplanted at 4 different ages (7, 14, 21 and 28 days old). Liquid fertilizer, 2 % Wuxal super (8:8:6), was applied at a rate of 200 ml/pot two weeks after sowing. Four replicates were used which were arranged in completely randomized design, the experiment was terminated 12 weeks after sowing and/or transplanting and was repeated twice.

For data collection the number of emerged *Striga* shoots/pot were counted weekly starting from the first parasite occurence. Dry weight of both, *Striga* and sorghum, was determined at harvest.

For statistical analysis, the data were subjected to an analysis of variance, where F-test was significant, comparison between means was performed using Duncan's Multiple Range Test at $P \leq 0.05$.

Results and Discussion

Underground stages of *Striga hermonthica* observed in root chambers

Transplanting of 14 to 28 days old sorghum seedlings reduced the germination of *S. hermonthica* seeds significantly compared to the directly sown crop (Table 1). This effect can be attributed to the fact that the production of the germination stimulant is confined to a restricted zone just near the root tip (Brown and Edwards, 1944). The number of *Striga* attachments were also significantly reduced in transplanted compared to directly sown crop (Table 2). In addition, negligible attachment was observed to sorghum roots that were developed before transplanting of the crop. Transplanting also resulted in a lower number of parasite shoots (Table 3). The resistance of older sorghum roots to *Striga* penetration could be due to the development of substances like lipids, phenols, suberin and lignin in the roots, which are deposited in the walls of epidermal, hypodermal and endodermal cells. These cell wall modifications were reported to appear at a short distance from the root apex of sorghum (Rolando *et al.*, 1992). However, sorghum roots that were 39 to 111 hours old were found to show no resistance to the parasitism by *S. asiatica* (Ramaiah *et al.*, 1991). The authors explained the non-significant effect of sorghum root age on the parasite incidence by the relative youth of the sorghum root. They also claimed that the presence of cell wall thickenings and lignification of the pericyclic cells in the host root tissue may delay the penetration process of *Striga* haustorial cells to the extent that it consequently fails to establish. The difference to the results of our study could be attributed to the difference in sorghum root age. In the presented study sorghum seedlings used were 7 to 28 days old, while Ramaiah and co-workers used sorghum seedlings that were less than five days old. With the increase of the age of sorghum roots more deposition of thickening and lignifying materials can be expected (Rolando *et al.*, 1992).

Introduction

Striga spp. (witchweed) are among the most serious pests of the main food crops, maize, sorghum, and pearl millet, in the semi arid tropics. *Striga hermonthica* (Del.) Benth. is considered the most important parasitic weed throughout the savanna areas of tropical Africa.

Subsistence farmers face a lot of difficulties in control of this parasite. Peasant farmers normally have only poor resources to invest in their fields and the gross value of their crops usually is too low to afford the use of expensive control strategies (Kroschel *et al.*, 1996). Reduction in crop loss and in the parasite's reproduction could be promoted if the host crop could be protected from *Striga* in its early stages. Transplantation of several weeks (4-6) old seedlings could be a way to protect the crop.

In Africa, transplantation is a traditional method of stand establishment. They usually use it after seedlings are thinned to make up gappy stands. The method is also used to compensate for the stand loss due to flood damage (Doggett, 1988). Maranthée (1991) reported successful transplantation of maize in Vietnam to reduce the crop period in the field to allow the cultivation of an additional crop.

Transplanting of cereal crops is a practice that is normally used in rice production. However, IITA (1993) reported on the beneficial effect of transplanting sorghum in relay with cowpea which significantly reduced *Striga* infection but at the same time did not result in improved sorghum yields. In field experiments in Germany, transplanting of maize and sugar sorghum was proved to be an effective method that led to a significant increase of crop yield (Scheffer, 1985, 1988) due to avoiding unfavorable environmental conditions, since the crop could be raised in other places then transferred to the field after suitable conditions prevail. Scheffer also reported that the method reduced the requirements for chemical plant protection and also with crop transplanting the peak of labour requirements could be avoided (Scheffer, 1988). The process could also be mechanized using transplanting machines (Scheffer, 1988).

The present work aimed to evaluate transplanting of sorghum as a method that may contribute to reduced damage in sorghum from *S. hermonthica* and its possible contribution to reducing the parasite's reproduction.

Materials and Methods

Influence of sorghum seedling age at transplanting on germination and underground development of *Striga hermonthica*

At weekly intervals, plastic pots (13x13x13 cm), filled with washed sand, were sown with 15 seeds per pot of sorghum var. Dabar-1-1-1 (harvested in Sudan, 1988) and thinned a week later to 10 plants/pot. The pots were placed under glasshouse conditions at alternating day/night temperature of 35-30/25-20C and 12 hour photoperiod. After four weeks sorghum seedlings were transplanted into root chambers (two seedlings per root chamber) containing preconditioned *S. hermonthica* seeds (collected in Sudan, 1989). Root chambers described by Vogt (1993) were used to observe the germination and underground development of the parasite. After transplanting the sorghum seedlings, the vessels were wrapped in black plastic and placed at an angle to avoid the host roots growing through the filter paper and were then transferred to a growth cabinet adjusted at 30/20C for 12/12 hours day/night temperature. The treatments were directly sown sorghum as the control and sorghum seedlings that were transplanted at 4 different ages (7, 14, 21 and 28 days old). Evaluation was done weekly using a dissecting microscope, parameters observed were germination, attachment and shoot growth of the parasite. All parameters were calculated based on the parasite seeds found within an area of 1.5 cm

V.10

TRANSPLANTING OF SORGHUM: A METHOD TO REDUCE YIELD LOSSES CAUSED BY THE PARASITIC WEED *Striga*

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ABSTRACT

The effect of sorghum root age on host/parasite relationship was investigated by transplanting sorghum seedlings (0 to 28 days old) to root chambers containing preconditioned *S. hermonthica* seeds. Germination and underground development of *Striga hermonthica* was found to be low in the transplanted compared to directly sown sorghum. The attachment of *Striga* seedlings to the host root declined with increase in sorghum root age. Older sorghum roots, formed before transplanting, were found to resist the parasite attack. In pot experiments transplanting of sorghum showed no significant effect on the number of emerged *Striga* plants at harvest compared to directly sown sorghum at transplanting, perhaps due to the very high *Striga* inoculum used. However, sorghum sown directly four weeks before transplanting resulted in a significantly higher parasite shoot dry weight compared to the treatment in which four weeks old sorghum seedlings were transplanted, although sorghum age in both treatments was the same. The method significantly reduced the damage caused by *Striga* to sorghum and increased the crop yield. Transplanting 3-4 weeks old sorghum seedlings significantly increased sorghum shoot yield to about 12 g/plant compared to about 8 g/plant of directly sown crop.

Table 3

EFFECTS OF UREA AND HERBICIDES ON SORGHUM STRAW YIELD

TREATMENTS	STRAW YIELD (T/HA)			
	1992		1992	
Sorghum variety	G/H	SRN39	G/H	RSN39
Untreated control	6.12	9.40	2.66	4.21
Urea (U)	9.32	11.01	6.88	9.04
Dicamba (D)	9.01	10.52	5.13	5.79
D + U	15.81	12.19	7.04	9.04
Chlorsulfuron (Ch)	12.92	9.53	8.41	7.90
Ch + U	16.17	12.31	13.63	10.39
Ch + D	13.25	12.29	9.77	7.40
Ch + U + D	16.04	12.64	12.93	12.27
S.E.±	1.03		0.903	

** Urea at 190 kg/ha, dicamba 300 g/ha. Chlorsulfuron 2.4 g/ha, G/H = Gadam.*

VI.9

CHEMICAL CONTROL OF *Striga hermonthica* ON SORGHUM

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ABSTRACT

Striga emergence was earlier and more intense on the local sorghum variety Gadam EL Hamam (G/H) than on the *Striga* resistant SRN39. Urea, dicamba and their combination effectively controlled the parasite (62 - 92%) on SRN39, but not on G/H. Chlorsulfuron and its tank mix with dicamba effected excellent control of *Striga* (77-100%) on both varieties. Straw and grain yield of sorghum were negatively correlated with *Striga* population density (r= - 0.56 to - 0.89). SRN39, untreated or treated with urea or dicamba, outyielded the corresponding G/H treatments. G/H treated with Chlorsulfuron (2.4 g/ha) significantly outyielded the equivalent SRN39 treatments. Chlorsulfuron treatments made to G/H at planting curtailed initial growth and reduced crop stand. Seed dressing with 8-naphthalic anhydride reduced chlorsulfuron toxicity and improved yield.

Additional key words - parasitism.

Table 1

UTILITY OF USING SYNTHASE INHIBITING HERBICIDES APPLIED TO MAIZE SEEDS FOR CONTROLLING *STRIGA HERMONTHICA* IN HERBICIDE RESISTANT MAIZE (AVERAGE OF TWO SITES IN KENYA, 1995)

TREATMENT		MAIZE		STRIGA		
Herbicide	Rate (gm a.i./ha)	Stand[a] (no./plot)	Yield (gm/plot)	8 Weeks (no./plot)	12 Weeks (no./plot)	Growth[b] (12 weeks)
Controll	0	13.5	179	24.2	39.4	5
Imazapyr	15	13.0	619	2.7	18.7	2
Imazapyr	30	11.9	538	2.5 c	32.4	1
Imazathepyr	70	9.9	399	19.2	22.0	3
Imazathepyr	140	9.2	218	3.4	23.7	2
Chlorsulfuron	10	10.0	307	5.5	16.0	2
Chlorsulfuron	20	10.7	456	4.7	12.4	1
Rimsulfuron	15	11.7	355	29.2	33.2	5
Rimsulfuron	30	8.4	249	14.5	21.0	4
Halosulfuron	60	8.5	364	4.8	10.0	4
Halosulfuron	120	8.5	260	7.7	35.3	3
Metsulfuron	5	7.5	215	5.0	17.5	3
Metsulfuron	10	6.2	117	5.2	13.7	2
Sulfometuron	50	3.8	86	0.5	7.4	2
Sulfometuron	100	3.2	86	0.0	8.4	1
LSD 0.05		2.5	77	17.3	28.7	–

[a] *two weeks after emergence, plot size = 3.75 m^2.*

[b] *Striga growth was assessed visually, where: 0 = no emergence; 1 = small plants, no flowering; 2 = medium plants, some flowering; 3 = larger plants, full flowering; 4 = large plants, some capsules; 5 = large plants, full capsules.*

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Discussion

These data demonstrate for the third season in a row that selected herbicides with known ALS inhibiting activity can be effectively applied directly on maize seed in the planting hole to provide early season *Striga* control. PH 3245 IR, which was specifically developed for use with imazapyr and imazathepyr in the USA, showed good tolerance to a wide-range of the herbicides, particularly at their lower rates of application. However, sulfometuron was particularly phytotoxic at both rates of application.

Striga control by drenching the seed with solutions of herbicides was affected by herbicides and their rates. With the exception of rimsulfuron, all herbicides provided some early-season *Striga* control. However, for treatments such as sulfometuron, metsulfuron and halsulfuron, which were somewhat phytotoxic to the maize, some of the apparent *Striga* control must be attributed to the reduced host growth, as plots with reduced plant stand and vigor, had fewer sites for parasitism to occur. At Kibos *Striga* control generally persisted longer resulting in higher maize yield. The prolonged control of *Striga* at Kibos could have been caused by better persistence of the herbicides or by unfavorable environmental conditions for *Striga* development during this period. Ransom and Odhiambo (1995) reported that rainfall pattern can significantly affect the timing and intensity of *Striga* parasitism.

Except in the most phytotoxic treatments, maize grain yield relative to the untreated control was increased with herbicide application. As we have seen in two previous seasons (Ransom *et al.*, 1995), imazapyr provided the best control and was the least phytotoxic to PH 3245 IR. Chlorsulfuron, in this experiment, also looks promising in protecting maize from *Striga*. The early season control provided by imazapyr, which delayed emergence of *Striga* for at least 4 weeks, imparted substantial yield advantage compared to the control. *Striga* exerts a potent phytotoxic effect on the host before it emerges from the soil and this effect appears to be cumulative (Ransom *et al.* 1996). Therefore, even a slight delay in parasitism can result in dramatic increases in yield of the host crops (Ransom and Odhiambo, 1992; Berner *et al.*, 1995).

Imazapyr and chlorsulfuron applied to maize with target site resistance show considerable promise as a means of protecting maize from *Striga*-related yield losses. Furthermore, by improving yields by delaying parasitism, farmers are provided with more resources and incentives to control the reproduction of *Striga* later in the season. Since farmers will not be required to purchase and calibrate sprayers, seed dressing herbicides should be readily adopted by them. Farmers in Kenya are used to purchasing and planting certified maize seeds which are treated against other pests. An additional advantage is that this technology will still allow maize to exude germination stimulants into the rhizosphere, thereby inducing germination of *Striga* seeds, further depleting the *Striga* seed banks when coupled with a program of stopping *Striga* reproduction and of stopping the introduction of new seed from off-farm sources.

PH 3245 IR did not yield well in this experiment as it was not well adapted. The data for both the *Striga* counts and the maize yield must be viewed with some caution, as a better adapted more vigorous genotype might have not only more yield potential, but provide more sites for parasitism. Better adapted materials with genes for herbicide resistance are needed in order to confirm the value of seed-dressing with these herbicides for *Striga* control. We are currently working on developing better adapted germplasm with ALS resistance. Additional research is needed on refining the rates of application, developing seed dressing techniques, determining the mode of action of the herbicides, and developing strategies for delaying herbicide resistance. Given the known frequency of resistance genes to ALS herbicides, we expect that in the first year, five resistant *Striga* plants/ha will emerge (Gressel *et al.*, 1996). A regime of rouging will be needed to preclude the rapid build-up of resistance.

infested with *Striga hermonthica*. PH 3245 IR, a maize hybrid commercially available in the USA, which has homozygous target site resistance to imidazolinone herbicides, was used in both experiments. Plots consisted of two 2.5 m long rows with a 75 cm spacing. Within these rows, maize was planted two seeds per hill, with hills spaced at 50 cm. Seven herbicides within the imidazolinone and sulfonylurea herbicide groups were applied to the seed in the planting hole as a 1 ml aqueous solution. The list of herbicides and rates used is found in Table 1. The rates utilized were based on the rates that were found to have some post-emergence efficacy in IR maize in the USA (personal communication, T. English) or from published rates for other uses. The experimental design was a randomized complete block with 3 replications. No fertilizer, insecticide or fungicide was applied and all weeds except *Striga* were removed by hand.

Maize stand establishment was determined about 2 weeks after planting. *Striga* counts were made on the entire plot every 2 weeks beginning when the first emerged *Striga* was noted (about 6 weeks after planting). A visual rating of *Striga* growth was made at 12 weeks after planting to appraise their potential to reproduce. Grain yield was obtained by harvesting the entire plot, and was adjusted to 15% moisture. These data were subjected to analysis of variance (ANOVA) combined across locations and means were separated using the least significant difference test at the 5% level of probability.

Results

For the variables measured in these experiments, there was no significant location by treatment interaction, so all data are presented as a mean of the two locations. Locations differed significantly, however, in number of emerged *Striga* at 12 weeks and in maize yield (separate data not shown). Averaged across all treatments there was significantly more *Striga* at Alupe (7.7/m^2) than at Kibos (2.9/m^2) at 12 weeks. Conversely, the average yield at Kibos (1050 kg/ha) was greater than that at Alupe (520 kg/ha).

Maize stand was significantly reduced compared to the untreated control by a number of treatments (Table 1). Sulfometuron was the most damaging to maize of the herbicides included, followed by metsulfuron and halsulfuron. Stand establishment was not affected by either rates of imazapyr or chlorsulfuron nor by the lower rate of rimsulfuron. There was good uniformity in distribution of *Striga* within both experiments. There was a relatively large increase in the number of *Striga* between the 8 and 12 week counts at Alupe (2.2 to 7.7 plants/m^2) compared to Kibos (2.4 to 2.9 plants/m^2) (data not shown). There were significant differences between treatments in the number of *Striga* supported 8 weeks after planting (Table 1). Rimsulfuron, a herbicide with very short persistence, did not control *Striga* at the rates included in the experiment. The best early season control was achieved with both rates of imazapyr and the highest rate of sulfometuron, although the latter can be explained by poor maize plant stand. By 12 weeks after planting there was an increase in *Striga* numbers in all treatments. However, the size of the *Striga* was very reduced by imazapyr, chlorsulfuron and sulfometuron, so much so that there was little or no *Striga* seed set (Table 1).

PH 3245 IR was poorly adapted to Kenya, as it was developed for and marketed in the corn belt region of the USA. PH 3245 IR was susceptible to northern corn leaf blight (*Setophaeria turcica*) and maize streak virus which were prevalent within the experiments. These diseases did not affect all plots uniformly, making an accurate assessment of treatment effects on yield difficult. In general, yields were very low relative to the potential for maize in this area. Nevertheless, there were significant differences between treatments (Table 1). Treatments which were partially phytotoxic to maize, such as metsulfuron and sulfometuron or that allowed for early season *Striga* parasitism, such as rimsulfuron generally had the lowest yields. The highest grain yield was achieved with imazapyr at 15 g a.i./ha.

VI.8

Striga hermonthica CONTROL WITH ACETOLACTATE SYNTHASE INHIBITING HERBICIDES APPLIED TO MAIZE SEED WITH TARGET-SITE RESISTANCE

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ABSTRACT

Striga spp. are more damaging when they infect the crop early in the season. Field experiments were conducted at two sites in western Kenya to evaluate several herbicides with acetolactate synthase (ALS) activity, for their ability to provide early season *Striga hermonthica* control when applied to the seed of maize. In the planting holes, seed of PH 3245 IR, a hybrid with ALS target-site resistance, was drenched with a 1 ml of the solution containing the herbicides. Imazapyr, chlorsulfuron, and rimsulfuron were the least and sulfometuron the most phytotoxic to maize at the rates used. Except for rimsulfuron, all herbicides provided some early season *Striga* control, with imazapyr and chlorsulfuron as the most effective at rates that were not phytotoxic to maize. Because PH 3245 IR was not well adapted to western Kenya it did not yield well. Nevertheless, imazapyr at 15 and 30 g a.i./ha and chlorsulfuron at 10 g a.i./ha provided significantly higher yield than the untreated control. The excellent yield from treatment with imazapyr at 15 g a.i./ha confirms the potential value of early season *Striga* control for protecting crop yields from *Striga* damage. Application of imazapyr to maize with target site resistance shows promise as a technology for reducing *Striga*-related yield losses for small-scale farmers in Africa.

Introduction

Crop losses, attributable to a specific pest, is a function of the area the pest infested. *Striga*, and other parasitic weeds, infest vast areas in Africa, the Old World Tropics, and throughout much of the world. Significant crop losses may occur within these infested areas. However, there still remains vast areas of potential habitat for these parasitic pests that are not yet infested. It is to the protection of these noninfested areas that I wish to focus our attention. We as weed scientists need to take a lesson from our entomology and pathology peers who are mindful of the role of prevention in the holistic management of a pest. They have long used regulatory authority, sanitation and mitigating treatments to prevent the movement of pest from infested to noninfected sites. This same prevention philosophy can be used is as effective management strategy against the movement of parasitic weeds. Movement prevention procedures should be employed to thwart the spread of parasitic weeds from field to field as well as from country to country.

Short Distance Movement

The most commonly recognized movement of a pest is within a field or from one field to another. Some of the mechanisms for movement of *Striga* include: (I) natural seed dispersal; (2) movement with commodities; (3) relocation by people, animals and equipment; (4) through tillage operations and (5) by the use of contaminated crop seeds. (Berner *et. al.* 1994). Biological factors which contribute to the dispersal of *Striga* include: very high seed productivity, the minute size of the seed, and an electrostatic charge that causes them to adhere to other seeds, commodities, soils and equipment. In addition, the long survival of parasitic weed seeds extends the period of time during which these seeds may move to and survive in a new habitat (Bebawi, *et.al.* 1984).

Some procedures and precautions that individuals can use to prevent the movement of *Striga* seeds from infested to non infested sites include the following: (1) prevent plants from producing viable seeds. No seeds above the soil level drastically reduces the potential for spread; (2)clean equipment and tools to insure that they are free of contaminated soil before transporting them to a noninfested field; and (3) planting crop seeds that have been harvested from noninfested fields or has been cleaned (Eplee *et.al.*, 1991). Common sense logic in preventing the movement of parasitic weed seeds is the key to protection. The propensity for the movement of seeds is relative to the size and intensity of an infestation. If seed populations can be reduced through a variety of common sense cultural practices, the likelihood of spread can be reduced. However, the most important element in thwarting movement of parasitic weeds is education. Until farmers and professional agriculturists understand and recognize the consequences of allowing the spread of *Striga*, there will be little incentive to prevent spread onto or within their farm. For farmers who do not yet have an infestation of a parasitic weed, proactive prevention is their best management strategy. The next logical strategy is the destruction of incipient and small infestations before seed production. Like a small fire, small infestations grow, reproduce (profusely) and spread (often exponentially) to unmanageable and devastating proportions.

Long Distance Movement

Long distance movement of a pest, such as *Striga*, constitutes a major threat to regional, national and international agriculture. The primary vector for long distance movement of *Striga* is through contaminated shipments of seeds, commodities and equipment. We have also intercepted *Striga* being imported into the USA as a medicinal herb. It is unknown how *Striga asiatica* (L). Kuntze was moved from Africa to the USA, but it was almost certainly inadvertently transported by man through trade activities. The consequence of this unintentional movement of *Striga* has been 40 years of intense eradication effort and the expenditure of over 200 million dollars of taxpayer money. And this effort is not over yet. This, and countless other pest

Table 1

EFFECT ON PARASITE GROWTH OF GLYPHOSATE APPLIED OVER ON VIRGINIA TOBACCO PLANTS (162 DAYS AFTER TREATMENT)

Glyphosate		Nb. broomrape/ tobacco plant (total)	Broomrape fresh weight (g/tobacco plant)	Broomrape shoots/ tobacco plant (emerged)	Parasite total lenght (cm)/ tobacco plant
108 dat (1)	129 dat				
100	100	8.0	85.9	0.2	10.1
100	200	5.6	70.3	0.1	6.6
200		4.9	38.9	0.1	4.3
200	300	3.0	34.9	0.0	2.5
Control (parasitized)	22.4	474.1	7.3	242.9	

(1) dat: days after treatment.

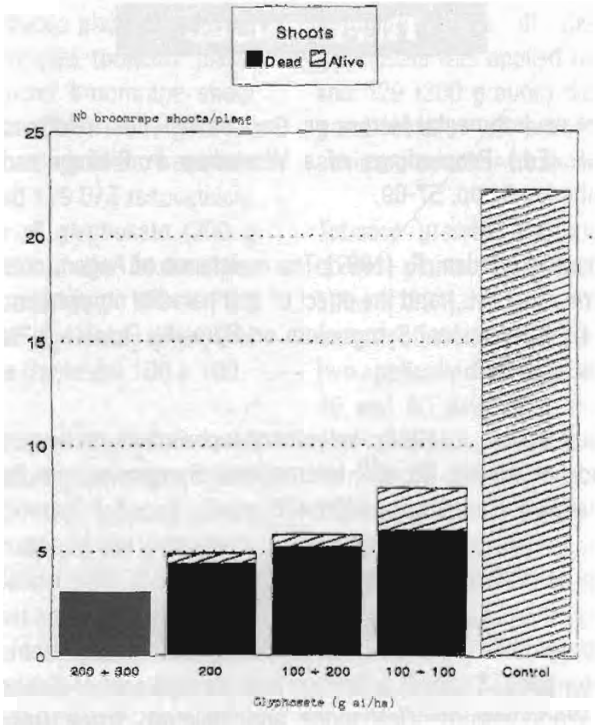

Figure 1

Parasitism of *Orobanche cernua* on Virginia tobacco plants sprayed with glyphosate.

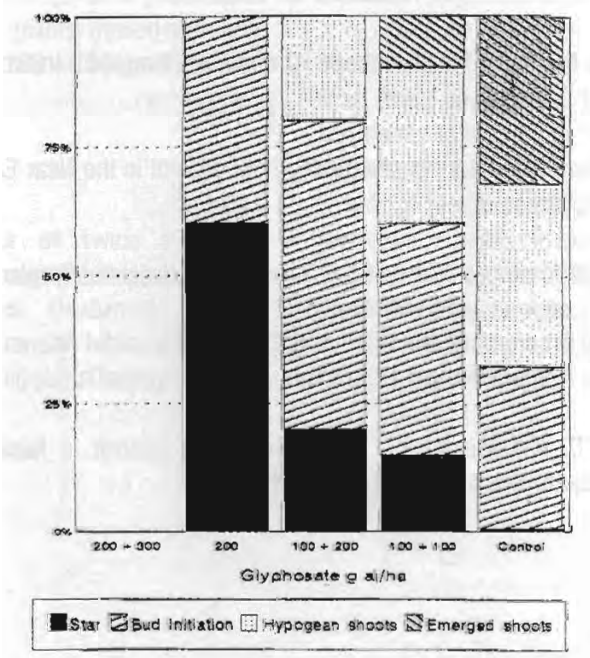

Figure 2

Post emergence application of glyphosate on parasitized Virginia tobacco plants. Proportion of different phenological stages of the parasite.

Table 1

EFFECT OF GLYPHOSATE ON TOBACCO AND *OROBANCHE CERNUA*

Treatments dosage (g a.i./ha)	DAT*	TOBACCO Plant height (cm)	Leaves/ plant	Leaf-yield Green (kg/ha)	Leaf-yield Cured (kg/ha)	*O. CERNUA* Plants infested** (%)	Spikes/ plant	Green wt. spikes/ plant**
37	30 & 35	125.7	24.0	6496	1261	32.9(29.5)	1.19(1.42)	3.56(13.0)
	30 & 40	156.8	27.8	9133	1439	32.1(28.3)	1.09(1.19)	3.61(15.3)
	35 & 40	128.8	24.6	7753	1057	28.7(23.6)	1.88(3.57)	4.16(18.0)
	35 & 45	151.3	27.5	10606	1599	37.5(37.2)	1.95(4.00)	7.25(27.7)
	40 & 45	146.5	26.5	10677	1556	40.5(40.5)	2.40(5.90)	6.00(37.5)
	40 & 50	157.7	27.4	10223	1496	44.1(48.5)	2.49(6.69)	5.62(32.1)
60	30 & 35	107.3	22.9	4781	807	22.8(15.4)	1.23(1.72)	2.57(7.0)
	30 & 40	117.2	22.2	6603	1081	33.3(30.5)	1.16(1.70)	2.29(6.1)
	35 & 40	94.7	21.5	4301	743	26.3(20.6)	1.00(1.10)	2.25(5.9)
	35 & 45	115.3	23.9	5826	929	30.4(27.0)	1.52(2.79)	2.82(8.9)
	40 & 45	123.2	24.7	7504	1143	30.5(25.9)	1.38(2.32)	3.65(14.2)
	40 & 50	147.5	27.8	9211	1323	31.2(27.5)	1.83(3.44)	2.98(11.2)
Control		172.0	28.8	11566	1894	65.4(78.8)	3.26(12.04)	8.62(81.8)
C D	5%	36.32	4.40	2899	497	11.4	0.802	1.806
	1%	49.21	NS	3930	671	15.4	1.087	2.447
CV %		16.06	10.3	21.37	23.49	19.3	27.65	26.09

** DAT - days after transplanting.*

*** Data transformed to angular transformation (original data in brackets).*

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glyphosate treatment. Researchers in Spain (García-Torres and López-Granados, 1991), ICARDA (Saxena *et al.*, 1994) and Egypt (Saber *et al.*, 1994) found similar results when using the same three herbicides against *O. crenata* on faba bean.

Hand pulling of orobanche shoots did not result neither in seed yield increase nor in improving the yield components. The damage caused by underground stages of the parasite seems to be as important as the emergent shoots in affecting considerably and negatively the yield. Kharrat *et al.* (1994) found that the non-emergent orobanche affect the growth and the seed yield of faba bean regardless of the number and biomass of emergent broomrapes.

It could be concluded that *O. foetida* could be controlled by pre-emergence application of imazethapyr or imazaquin at the rate of 0.08 kg a.i./ha. Integrated control of the parasite could also be achieved in heavy infested fields by using one of these herbicides, moderately resistant lines and delayed planting as already shown (Kharrat and Halila, 1994).

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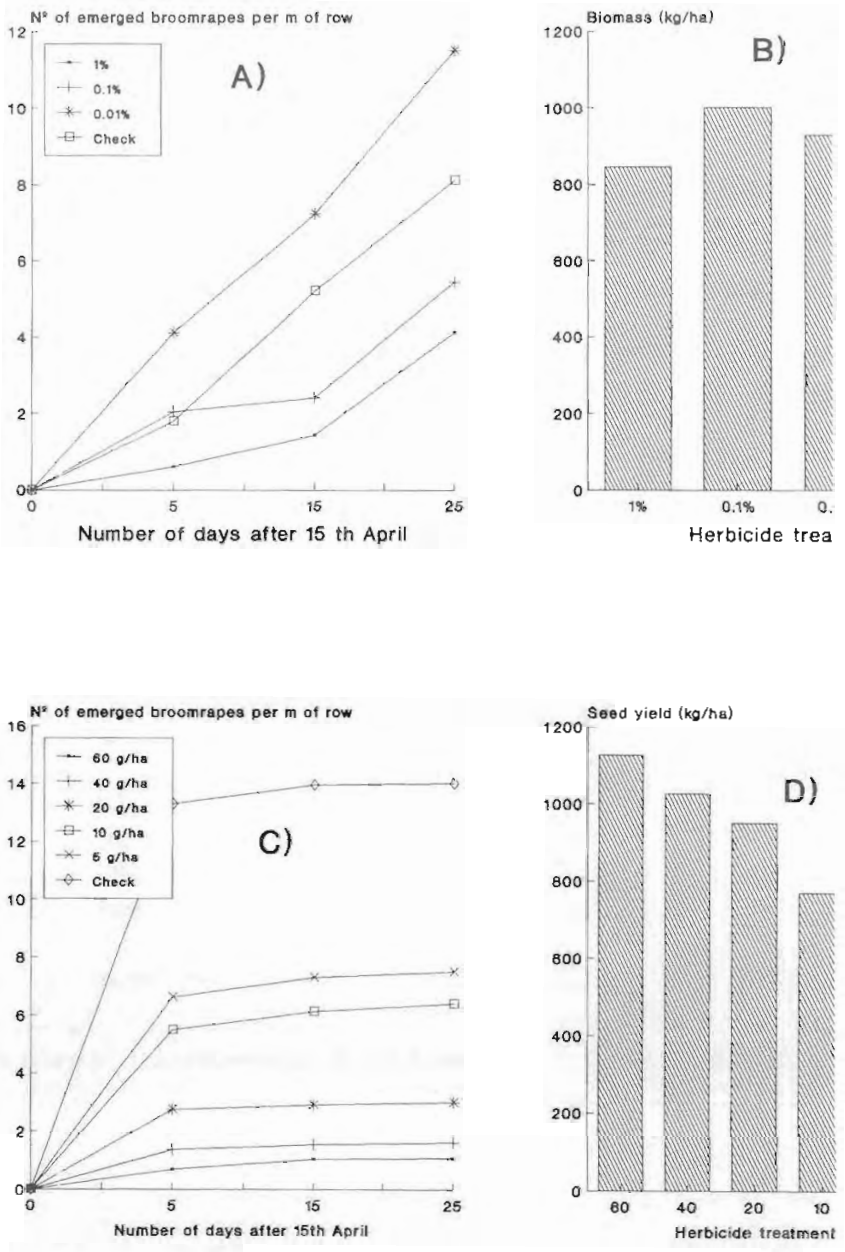

Figure 1

Effect of chlorsulfuron on *O. aegyptiaca* development in PE bags Applied at preconditioning (a), germination (b) and tubercles (c) stages. Attachment (radicle penetration into root epidermis), parasites (tubercles, spiders, stems). DAA-days after application. Bars in each observation period, followed by a different letter days significantly at *P=0.05* according to Duncan's multiple range test.

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days total count indicated significant reduction of the number of parasites on tomato roots by 70 and 90% at 2.5 and 12.5 mg/l respectively. Rimsulfuron at 0.25 mg/l showed no effect on *Orobanche* development.

Rimsulfuron at all concentrations was not phytotoxic to tomato plants.

Discussion

Sulfonylurea herbicides applied at low concentrations (0.1 mg/l) on *O. aegyptiaca* seeds at the preconditioning and germination stages reduced radicles length. These findings support those of Westwood et al. (1995), that chlorsulfuron applied at 0.01 M (equal to 0.0036 mg/l) during Orobanche seed germination decreased radicles length to 75% as compared to untreated control, while other sulfonylurea herbicides were effective only at 0.1 M. The observed effect of sulfonylurea herbicides on *Orobanche* development can be explained by their mode of action on amino acid synthesis, causing inhibition of cell division and growth. Chlorsulfuron up to 1 mg/l inhibited soybean cell growth in culture, without loss in cell viability (Blair and Martin, 1988).In our polyethylene bags experiments, chlorsulfuron and triasulfuron at 0.01 mg/l and rimsulfuron at 0.25 mg/l did not stop Orobanche development, but caused abnormal growth in part of the parasites. Chlorsulfuron and triasulfuron at 0.1 mg/l and rimsulfuron at 2.5 mg/l, applied at different *Orobanche* development stages halted the parasitism process. The herbicides in this study affected *Orobanche* in all developmental stages, but the effect was strongest, when parasites were small, emphasizing the importance of application timing for *Orobanche* control. The effect diminished 26-28 days after herbicide application, probably due to herbicide degradation (Blair and Martin, 1988). In our study chlorsulfuron was more potent to Orobanche and tomato than triasulfuron and rimsulfuron. These results are in accordance with Garcia Torres et al. (1994) that chlorsulfuron is more effective than triasulfuron in controlling O.cumana in sunflower. Our findings support previous field experiment results in which the correct repeated application timing was a limiting factor in *Orobanche* control (Kleilfeld et al.1994; 1996).

The results obtained in this study provide better understanding of the precise timing and rate of sulfonylurea herbicide application, needed for improved *Orobanche* control .

Acknowledgments

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concentration ranges: triasulfuron at 0.01-10.0 mg/l a.i., chlorsulfuron at 0.01-10.0 mg/l a.i. and rimsulfuron at 0.25-12.5 mg/l a.i. Treatments were replicated 5 times (5 bags). Experiments duration was 7 weeks.

Resutls

Petri plate experiments. All three herbicides at all concentrations (0.1-10.0 mg/l), applied at the germination stage, reduced radicle elongation by 45-56% as compared to the untreated control (Table 1). At the lowest concentrations (0.1 mg/l), radicle appearance was not affected. High concentrations (1-10 mg/l) significantly augmented the percentage of necrotic radicles and the damage to radicles, as was observed at the 10.0 mg/l chlorsulfuron treatment that caused 81% necrotic radicles (Table 1).

Polyethylene bags experiments. Sequence of events of *O. aegyptiaca* development in polyethylene bags was as follows: 3-4 days after GR_{24} injection, seed germination rate reached 60-80%; 1-3 days after germination the first attachments appeared; 10-15 days after germination, maximum attachments were observed (40-80 per bag without GR_{24} stimulation and 160-260 per bag with GR_{24} stimulation). Ninety percent of the attachments developed into tubercles and spiders and a few into stems and flowering inflorescences. New germinating seeds continued to attach to the host roots for a period of one month but at a low rate.

Effect of Chlorsulfuron on Orobanche development. When chlorsulfuron was applied at the germination stage, damage to *Orobanche* radicles was detected 5-7 days after herbicide application . Ten days after application a decline in the number of attachments and tubercles was observed in all treatments. The parasitism process continued, but many of the attached radicles turned necrotic (Fig.1b). Few attachments developed into tubercles, but these turned necrotic after one week. The same effect was observed, when chlorsulfuron was applied at the preconditioning stage (Fig.1a). When the herbicide was applied at tubercle stage, 80% of the tubercles turned necrotic after 10-15 days (Fig.1c). Eighteen -30 days after application, big spiders and stems turned necrotic too. Development of new attachments was not completely stopped, but the attachments failed to develop into tubercles. Application of chlorsulfuron (0.1 mg/l) at the three different parasite developmental stages prevented further development of young tubercles. New attachment formed after 21 days and continued to grow into tubercles, but these were deformed, having stubbed papillae. This phenomenon is characteristic of sulfonylurea herbicides damage to Orobanche as exhibited throughout this study. Chlorsulfuron and triasulfuron at 0.01 mg/l did not reduce the number of attachment and parasites on tomato roots, but 10-20 % of the tubercles acquired a strange form. High concentrations of chlorsulfuron (1 and 10 mg/l) were phytotoxic to tomato plant.

Effect of Triasulfuron on Orobanche development. Triasulfuron applied at different parasite development stages significantly reduced the total number of parasites attached to tomato roots (data not presented). The damage caused by triasulfuron at 0.1 mg/l as compared to chlorsulfuron at the same concentration was minor and disappeared after 34 days , so that the number of parasites found on tomato roots after this period was the same as on the untreated control. High concentrations of Triasulfuron (10 mg/l) were phytotoxic to tomato plant.

Effect of Rimsulfuron on Orobanche development. Rimsulfuron applied at preconditioning stage, delayed *Orobanche* development. The inhibition disappeared after 28 days at 2.5 mg/l, and after 35 days at 12.5 mg/l (Table 2). Rimsulfuron at 2.5 and 12.5 mg/l applied at germination or tubercle stages, delayed Orobanche development, and attachments and tubercles appeared necrotic. Four weeks after herbicide application most of the new attachments and tubercles began to appear healthy. After 28

Table 2

RESPONSE OF TOMATO AND *O. AEGYPTIACA* TO CHLORSULFURON AND TRIASULFURON APPLIED POSTEMERGENCE VIA DRIP IRRIGATION IN THE FIELD, GESHER HA'ZIW 1994 (EXPT. 2)[1]

		TOMATO YIELDS (KG/10 M)			
Herbicide	Rate (g/ha)	Red fruits	Green fruits	Total	Foliage
Chlorsulfuron	3.75	10.7 a	17.0 a	27.7 a	7.6 a
	7.50	9.4 a	20.8 a	30.2 a	8.6 a
	11.25	12.6 a	19.3 a	31.9 a	8.1 a
Triasulfuron	7.50	13.7 a	17.4 a	31.1 a	9.5 a
	11.25	10.9 a	15.8 a	26.7 a	7.5 a
	15.00	10.3 a	18.2 a	28.5 a	6.8 a
Untreated		10.3 a	16.4 a	26.7 a	8.0 a
		O. aegyptiaca infestation (shoots/20 m)			
		June 24	*July 1*	*July 8*	*July 15*
Chlorsulfuron	3.75	3.1 a	7.3 a	23.9 b	51.7 a
	7.50	1.6 a	5.9 a	14.0 b	42.7 a
	11.25	2.6 a	7.0 a	16.3 b	39.1 a
Triasulfuron	7.50	4.9 a	11.7 a	26.7 b	52.1 a
	11.25	3.1 a	12.1 a	30.9 b	52.7 a
	15.00	2.4 a	7.9 a	21.7 b	39.1 a
Untreated		2.7 a	10.7 a	56.8 a	64.3 a

[1] *Tomato transplanted on May 2, chemigated on May 25 and and harvested on July 21. Means in each column followed by the same letter do not differ significantly (P=0.05).*

Table 1

RESPONSE OF TOMATO AND *O. AEGYPTIACA* TO CHLORSULFURON AND TRIASULFURON APPLIED POSTEMERGENCE DIRECT TO THE SOIL, IN POTS, NEWE-YA'AR 1994 (EXPT. 1)[1]

			TOMATO			*OROBANCHE*
Herbicide	Rate	*Orobanche*	Height	Fresh weight	Dry weigh[1]	
Chlorsulfuron	3.75	+	25.0 ab	17.5 a-c	4.55 a	0 b
		-	28.4 a	20.3 ab	3.82 ab	
	7.50	+	21.4 ab	18.6 ab	3.56 ab	0 b
		-		23.0 ab	17.1 a-c	3.20 a-c
	15.00	+	16.8 b	12.6 c	2.34 bc	0 b
		-		13.8 b	8.6 cd	1.49 c
Triasulfuron	3.75	+	32.2 a	20.1 ab	3.63 ab	0 b
		-		30.6 a	22.5 a	4.72 a
	7.50	+	33.8 a	19.7 ab	3.65 ab	0 b
		-		33.0 a	18.7 ab	3.58 ab
	15.00	+	34.4 a	22.6 a	4.71 a	0 b
		-	33.0 a	21.6 a	4.27 a	
Untreated		+	25.4 ab	14.3 c	2.62 bc	22.8 a

[1] *Tomato transplanted in (+) = Orobanche infested or (-) = Orobanche free soil, on March 21. Means in each column followed by the same letter do not differ significantly (P= 0.05.)*

Introduction

The holoparasite, *Orobanche aegyptiaca* Pers. attacks and severely damages more than 30 food and ornamental crops in the Middle East (Parker and Wilson, 1986). Most measures being used for *Orobanche* control have been aimed at reducing the parasite seed bank in the soil of infested fields and have yielded inconsistent results. The most effective of these methods are prohibitively expensive (Foy *et al.*, 1989; Parker and Riches, 1993).

Only a few investigators have reported effective *Orobanche* control using systemic herbicides and even fewer reported effective and selective applications of soil residual herbicides (Foy *et al.*, 1989; Parker and Riches, 1993).

Recently soil applied sulfonylurea and imidazolinone herbicides were reported to show activity in *Orobanche* control, but low selectivity limits the rrange of crops to which they can be applied. (Parker and Riches, 1993; Garcia-Torres *et al.*, 1995). We have previously checked the effectiveness of rimsulfuron, a sulfonylurea herbicde with tomato selectivity in controlling *O. aegyptiaca* in pots, followed by field experiments and came to two conclusions: 1. The herbicide was found effective in controlling the parasite when applied to the tomato root zone and not through tomato foliage translocation. 2. Since rimsulfuron soil residual activity is short, repeated applications were necessary for *O. aegyptiaca* control throughout the season (Kleifeld *et al.*, 1994). As a result of these findings, we have tried in this study to apply soil-long residual sulfonylurea herbicides directly into the soil for *Orobanche* control in tomatoes, yet avoiding their direct contact with the tomato foliage.

Materials and Methods

Pot experiments

Two experiments were performed using Newe Ya'ar clay-loam soil (58% clay, 25% silt, 17% sand, 14% $CaCO_3$ and 2% organic matter; pH 7.1-7.2). The air-dried soil was uniformly mixed with 10 mg per kg *O. aegyptiaca* seeds previously collected from tomato hosts. Soil without *Orobanche* was used as a control. In 1994 (Expt. 1), 5 l plastic pots were used and in 1995 (Expt. 3), the pot capacity was 10 l. Tomato var. M-82 plants with 4-5 leaves were transplanted into the pots and grown in the greenhouse. The plants were sprinkler irrigated as necessary, except when herbicide treatments were applied by chemigation.

In Expt. 1 the herbicide dose for each pot was mixed in 1 l of water and was poured slowly on the soil surface. The single application was made 10 days after transplanting. In Expt. 3 the herbicide was mixed in 0.5 l water and the solution was sprinkled on the tomato foliage by using a small garden sprinkler, followed by sprinkling an additional 0.5 l water. Herbicide was applied in either a single treatment, 12 days after transplanting or in a split treatment applied 12 and 24 days after transplanting. Each treatment was replicated five times on soil with and without *Orobanche* seeds. Tomato plants were harvested when *Orobanche* shoots in the untreated pots started to develop seeds; at that time tomato plant height, foliage fresh and dry weights were measured. *Orobanche* inflorescenses were counted and after harvesting the host plant, the soil in the *Orobanche* contaminated pots was washed carefully through a 2 mm screen and the total number of parasites were counted.

Field experiments

Two experiments were conducted; in 1994 (Expt. 2) and in 1995 (Expt. 4) in Gesher Ha'Ziw on a clay loam soil (48% clay, 25% silt, 27% sand, 22% CaCO3 and 2% organic matter; pH 7.3). The experimental field was known to be heavily infested with *O. aegyptiaca*. In both years the field was plowed and leveled with raised beds (1.93 m width) and trifluralin was incorporated preplanting for annual weed control. Tomato var. M-82 was transplanted into the beds in two rows

VI.2

SELECTIVE CONTROL OF *Orobanche aegyptiaca* IN TOMATO WITH SULFONYLUREA HERBICIDES

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ABSTRACT

Soil treatments with the sulfonylurea herbicides chlorsulfuron and triasulfuron controlled or effectively inhibited *O. aegyptiaca* parasitizing tomatoes. The typical injury to tomato by these herbicides when sprayed postemergence was avoided by applying the herbicides by chemigation. Injection of the herbicides into the drip irrigation system in an *O. aegyptiaca* contaminated tomato field at 4-6 leaf stage did not damage the crop, but did not effectivly control *Orobanche*. However, when the herbicides were applied via a sprinkler irrigation, *Orobanche* control was excellent, while herbicide damage to young transplanted tomatoes was not significant thanks to the great dilution of the herbicide and immediate sprinkling with uncontaminated water. Split applications with half rates were more effective in decreasing *Orobanche* infestation and more selective to the crop than one application with a full rate. Triasulfuron appeared to be safer than chlorsulfuron on the tomatoes.

VI.1

HERBICIDE-TREATED CROP SEEDS FOR CONTROL OF *Orobanche* SPP.[1]

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ABSTRACT

New herbicide treatments for control of broomrape (*Orobanche* spp.) have been developed by seed immersion in commercial herbicide solutions or by seed coating, using some coating substances as a herbicide carrier. Greenhouse and field studies conducted in 1993-1995 have shown that imazethapyr applied to pea and faba bean seeds, imazapyr on lentil seeds, and propyzamide on sunflower seeds resulted in medium to high broomrape control. Herbicide rates vary with the crops, herbicide and method of application. The advantages of the herbicide-treated seeds for broomrape control as compared to conventional pre-emergence herbicide treatments are discussed.

1 Procedure used patented by CSIC (nº 94/02149, 94/02150, 94/02151, 94/02152).

VI.

Man and Parasites: Control

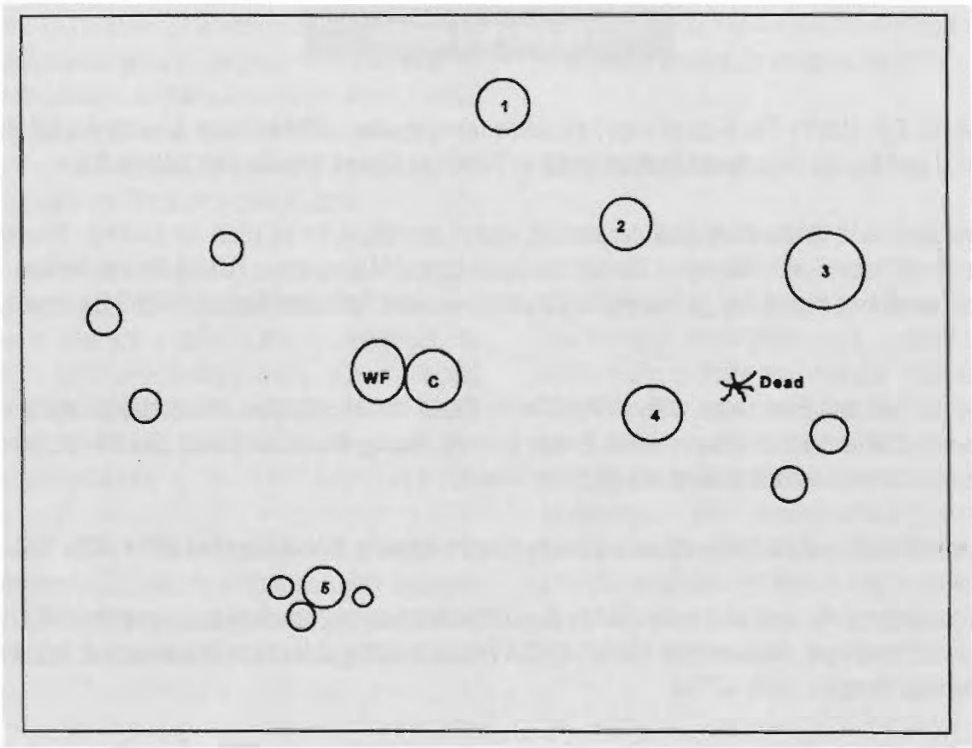

Figure 1

Map of candidate tree (C), source trees (numbered), a screening *Abies concolor* (WF), and other surrounding infected trees.

Table 1

DATA COLLECTED IN THE FIELD FOR THE TREES IN FIGURE 1

Tree	Age	Height meters	Diam cm.	Crown Ratio	Hawksworth Rating Upper	Mid	Lower	Total	Distance to Source
C	93	19.8	37	70%	0	0	0	0	
1		18.3	36	80%	1	2	2	5	< 5 meters
2		18.3	36	80%	1	2	2	5	< 2 meters
3		18.3	41	90%	1	2	2	5	< 6 meters
4		19.8	36	80%	0	1	2	3	< 2 meters
5		10.7	20	80%	2	2	2	6	< 5 meters

V.21

DWARF MISTLETOE RESISTANCE IN PONDEROSA PINE: SELECTION AND TESTING PROTOCOLS

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ABSTRACT

Western dwarf mistletoe (*Arceuthobium campylopodum*), a native pathogen, has developed infection levels incompatible with management objectives of many pine forests in the western United States. Studies of ponderosa pine (*Pinus ponderosa*) in Oregon and California have suggested that genetically inherited resistance mechanisms might be used to reduce infection to acceptable levels. Guidelines have been developed for the selection of potentially resistant candidate parent trees. Using these guidelines, 65 candidates and 10 highly susceptible controls were selected in 1994 and 1995. Wild stand seed has been collected from 40 of the candidates and all 10 controls to provide seedlings for eventual outplant tests and possible artificial inoculation. Earlier work in California resulted in six resistant candidates being identified. They were grafted into the Badger Hill Breeding Arboretum in 1965. These six clones and six suspected susceptible clones were artificially inoculated from 1993 to 1995 to identify differences in infection levels and to evaluate this as a future screening method. Patch grafting techniques are also being tried as a possible screening method. Controlled-cross seed from these six candidates, forming a half-diallel with some reciprocals, will provide first generation offspring for further testing.

Table 1

ANALYSIS OF VARIANCE IN A COMPLETE 9 x 9 SORGHUM DIALLEL EVALUATED FOR STIMULATION OF *STRIGA* SEED GERMINATION USING THE AGAR-GEL ASSAY WITH SOURCES OF *S. HERMONTHICA* FROM SMANKO (MALI) AND BENGOU (NIGER)

Source of variation	df	Mean square
Striga source	1	70.5 **
Sorghum genotypes	80	48.0 **
Parents	8	94.8 **
Parents *vs.* hybrids	1	6.4 *
Hybrids	71	43.0 **
GCA	8	292.0 **
SCA	27	16.6 **
Reciprocal differences	36	7.6 **
Sorghum genotype ˣ *Striga* source interaction	80	6.2 **
Parents ˣ *Striga* source	8	5.2
(Parents *vs.* hybrids) ˣ *Striga* source	1	1.9
Hybrids ˣ *Striga* source	71	6.4 **
GCA ˣ *Striga* source	8	4.1
SCA ˣ *Striga* source	27	6.0 *
Recipr. diff. ˣ *Striga* source	36	7.3 **
Experimental error[1)]	289	3.5

*1) Degrees of freedom reduced due to heterogeneity of error variances and deductions for missing values; *, ** Significant at the 0.05 and 0.01 probability levels, respectively.*

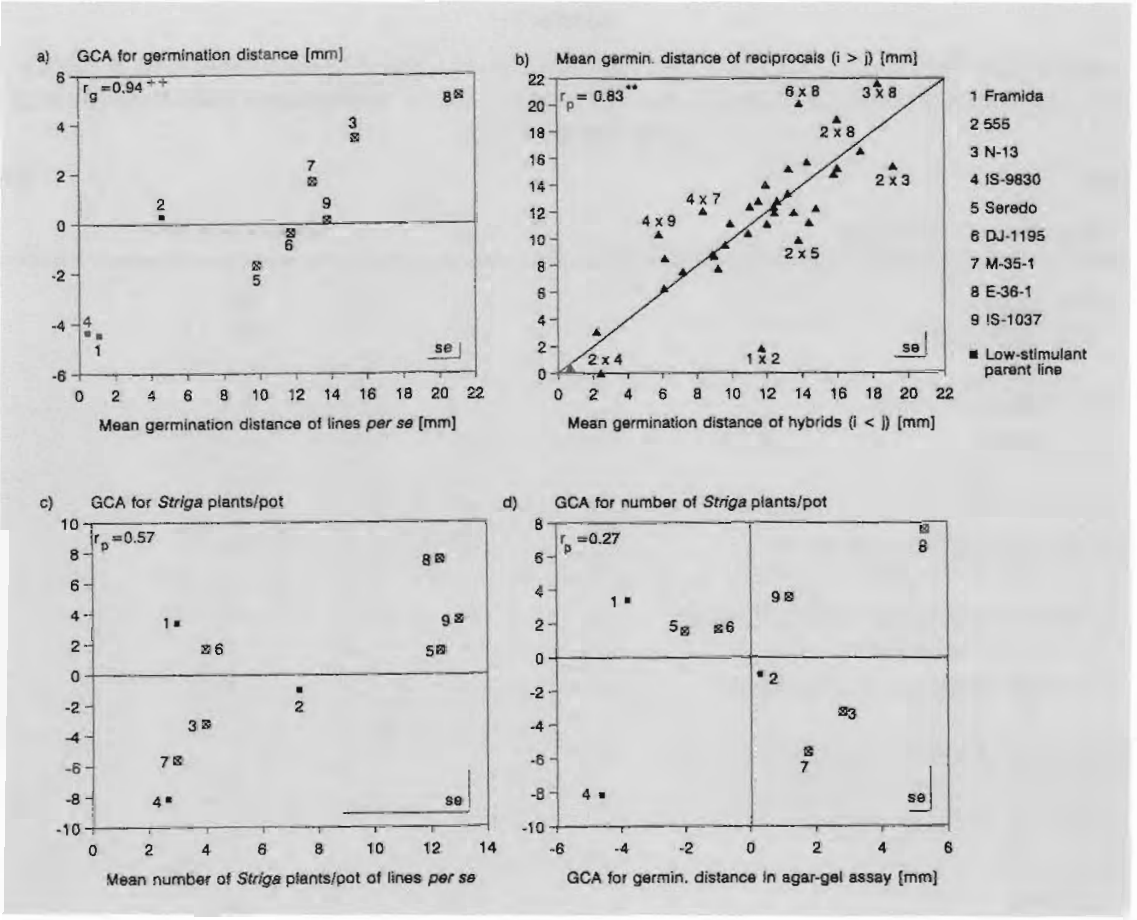

Figure 1

Relationship between (a) line performance an GCA and (b) hybrids and their reciprocal crosses for the germination distance in the agar-gel assay, averaged across two geographic sources of *Striga hermonthica*, and relationship between (c) line performance and GCA for the number of *Striga* plants per pot and (d) GCA for germination distance and number of *Striga* plants per pot; (r_g, r_p coefficients of genetic and phenotypic correlation, respectively; $^{+}$, $^{++}$ estimate exceeds its standard error once and twice, respectively, ** significant at P=0.01; se = standard error).

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V.20

QUANTITATIVE-GENETIC PARAMETERS FOR RESISTANCE TO *Striga hermonthica* IN SORGHUM

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ABSTRACT

A complete F_1 diallel involving nine selected sorghum [*Sorghum bicolor* (L.) Moench] cultivars and inbred lines was evaluated for stimulation of *Striga hermonthica* (Del.) Benth seed germination in the laboratory using the agar-gel assay with sources of *Striga* from Mali and Niger. The same genetic materials were planted in a pot trial in Mali to observe the number of emerged *Striga* plants per pot. Genotypes Framida, 555, and IS-9830 were classified as low-stimulant producers. Variation in hybrid performance was largely determined by general combining ability effects for both, the germination distance in the agar-gel assay and the number of *Striga* plants/pot. Specific combining ability (SCA) and reciprocal effects were significant only in the agar-gel assay. The sorghum genotype-*Striga* source interaction was consistent with instability of SCA and reciprocal effects across *Striga* sources for the germination distance. Estimates of broad sense heritabilities were 0.97 and 0.91 for the germination distances of lines and hybrids, respectively. Only a weak positive relationship existed between germination distance in the agar-gel assay and number of *Striga* plants in the pot trial. Although selection for low germination distance in the agar-gel assay among parent or early generation lines has a merit, it entails the danger of losing valuable materials with resistance mechanisms other than low-stimulant production.

Additional key words: *Striga* resistance, genetics, indirect selection.

Table 3

THE SUSCEPTIBILITY OF *O. GLABERRIMA* (CG14) AND *O. SATIVA* (WAB 56-104) PARENTS AND 47 PROGENY TO *S. ASPERA* AND *S. HERMONTHICA* EX COTE D'IVOIRE. MEAN EMERGED *STRIGA* NUMBER PER POT OF ONE RICE PLANT AT 134 DAYS AFTER PLANTING. ACTUAL AND TRANSFORMED DATA ARE PRESENTED

Line	*S. ASPERA*		*S. HERMONTHICA*	
	$\sqrt{x+1}$	Actual	$\sqrt{x+1}$	Actual
PARENTS				
CG14	–	0	–	0
WAB 56-104	3.8	16.3	3.3	10.5
Progeny				
WAB450-18-3-2	4.1	18.5	2.0	4.0
WAB450-24-2-17	3.0	9.5	2.8	7.0
WAB450-24-3-6	4.4	20.3	2.6	7.8
WAB450-25-1-9	4.3	20.8	2.5	6.3
WAB450-25-2-9	5.0	26.8	2.1	5.5
WAB450-25-3-9	3.8	14.8	2.0	3.5
WAB450-25-3-2	4.5	20.3	1.8	3.3
WAB450-29-3-16	2.8	8.3	2.7	7.5
S.E.	0.8		0.7	

Table 4

THE SUSCEPTIBILITY OF TWO *O. SATIVA* LINES AND *O. PUNCTATA* TO *S. ASIATICA* EX ZANZIBAR. NUMBER OF EMERGED *STRIGA* STEMS PER POT OF ONE RICE PLANT AT 125 DAYS AFTER PLANTING. ACTUAL AND TRANSFORMED DATA ARE PRESENTED

Line	$\sqrt{x+1}$	Actual
IR49255-BB-5-2	1,00	0
O. punctata	1,85	2,8
B3193F-165	3,07	8,8
S.E.	0,66	

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IR47255-BB-5-4. These also appeared resistant in pot culture to *S. hermonthica* collected from Dokaha, Cote d'Ivoire and Homa Bay western Kenya. All other *O. sativa* lines proved susceptible. Low levels of susceptibility to *S. hermonthica*, were observed among *O. glaberrima* accessions and a number which appeared completely resistant to *S. aspera* did not support emergence of *S. hermonthica* from Cote d'Ivoire. This pattern of response was confirmed in a subsequent pot trial (Table 1). The introduced *O. sativa* cultivars Iguape Cateto and IAC165, which are widely grown in northern Cote d'Ivoire, are highly susceptible in pot culture to both *S. aspera* and *S. hermonthica*, as is commonly observed in the field. Of other *O. sativa* accessions, the lowest susceptibility was observed for WAB0688 (T1), a traditional landrace. In a series of field trials in western Kenya, lines IR49255-BB-5-2 and IR47255-BB-5-4 supported less than 10% of the *S. hermonthica* dry weight observed on susceptible check lines (Harahap *et al.*, 1992). Resistance to *S. aspera* from Cote d'Ivoire and *S. hermonthica* from both West and East Africa was confirmed during the current study. A high levels of resistance to both *Striga* species from Cote d'Ivoire was demonstrated for *O. glaberrima* lines ACC102196, Makassa and IG10 although these did support the emergence of the *S. hermonthica* sample from Kenya. *Striga* did not start to emerge on these until between 13 and 21 days after it was first observed on the susceptible cultivar Namroo. *Striga* growth following emergence was also limited on the *O. glaberrima* accessions. On ACC102196, for example, mean dry weight of emerged parasite stems was 0.44 g at 96 days after planting, compared to more than 2.2 g on Namroo.

The very high levels of resistance, in terms of parasite emergence observed in pots, were not seen at parasite infested field sites in Cote d'Ivoire (Table 2). However, *S. hermonthica* emergence was delayed and total number of parasite stems were less on *O. glaberrima* lines, IR49255-BB-5-2 and IR47255-BB-5-4 compared to the check IAC165. This response was less evident at the *S. aspera* infested site. Here, due to the effects of *Striga*, there was a considerable difference in the growth of rice lines. IAC165 and the traditional *O. sativa* landraces T1 and M2 produced stunted plants from which little biomass was harvested in comparison to IR49255-BB-5-2, IR47255-BB-5-4 and *O. glaberrima* lines. Further field testing is needed to assess the yield of these lines at infested sites.

The resistance observed in pot culture of *O. glaberrima* CG14, to both *S. aspera* and *S. hermonthica*, was not expressed in the F7 generation of the progeny resulting from the cross of this line with the susceptible *O. sativa* line WAB56-104 (Table 3). In order to increase the fertility of the hybrids, it was necessary to backcross the F1 generation twice to the *O. sativa* parent. This process appears to have produced progeny with *Striga* susceptibility similar to the susceptible *O. sativa* parent rather than transferring resistance to the hybrids. This suggests that it would be difficult to use *O. glaberrima* as a source of *Striga* resistance in a breeding programme.

A sample of the red flowered morphotype of *S. asiatica*, collected from upland rice in Zanzibar, was also included in the initial pot screening trials. With the exception of IR49255-BB-5-2 and IR47-BB-5-4 all lines tested, including *O. glaberrima* types, supported parasite emergence indicating that cross resistance of rice lines to different *Striga* species is not general. *O. sativa* line B3193F-165, reported to be resistant to *S. hermonthica* in Kenya (Harahap *et al.*, 1992) was particularly susceptible to *S. asiatica* (Table 4). The resistance of IR49255-BB-5-2 was confirmed in this subsequent trial. The wild species *O. punctata* (sample from Zanzibar) supported emergence of the red flowered form of *S. asiatica* in pot culture. In the single test conducted to date, this wild rice appeared resistant to the yellow flower morphotype of the parasite (sample from Cote d'Ivoire). These results should now be verified at *S. asiatica* infested field sites.

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Introduction

Striga species affect rainfed rice in the guinea savannah of West Africa, and while infestations tend not to cause widespread losses, localised infestations can be severe. *S. aspera* (Willd.) Benth. and *S. hermonthica* (Del.) Benth. have been recorded in upland rice in Cote d'Ivoire and Nigeria (CIDT, 1987; Parkinson, 1985). *S. aspera* occurs on hydromorphic soils, often favoured for rice production, while *S. hermonthica* is more commonly observed at free-draining sites. *S. hermonthica* has also been reported as a problem in Gambia (Carson, 1989), Burkino Faso (Ouedraogo, 1989), Mali (Anon, 1989) and Cameroon (Johnson, personal communication). In East Africa the species parasitises rice crops in the Lake Victoria basin area of Kenya (Harahap *et al.*, 1992) and Tanzania (Mbwaga, 1993). The red flowered morphotype of *S. asiatica* (L.) Kuntze is the problem species in rice on Madagascar and other Indian Ocean Islands (Reneaud, 1978, Fujisaka, 1990), while we have seen the yellow flower form of the species parasitising rice in Cote d'Ivoire.

Striga species tend to affect rice where soil fertility is low, and in consequence the problem is largely associated with resource poor farmers who do not apply mineral fertiliser. The development of host plant resistance is likely to be the most successful means to establish control of the parasite. To investigate the feasibility of this approach a study was undertaken of the variability in susceptibility to *Striga* species within cultivated *Oryza* species. Resistance is available in *O. sativa* to *S. hermonthica* from Kenya (Harahap *et al.*, 1992). Known resistant lines were included in glasshouse screening of *O. sativa* landraces from West Africa, introduced varieties and lines from the breeding programme at West Africa Rice Development Association (WARDA). Accessions of the African rice species, *O. glaberrima*, were also studied. Hybrids of *O. glaberrima* and *O. sativa* have been developed at WARDA in an effort to combine a range of resistances to biotic stresses with the yield potential of *O. sativa*. The *Striga* susceptibility of progeny from one such cross was also investigated.

Materials and Methods

The *Striga* susceptibility of rice germplasm was assessed in a series of screening trials undertaken in a glasshouse in the UK. Three plants of each entry were raised, one per replicate 1 litre capacity pot (127 mm diameter). The glasshouse was maintained at a minimum 25^0C and maximum 30^0C by day and minimum 20^0C at night. The pots were filled with a mixture of loam, peat and grit/sand (3:2:1) to which approximately 500 germinable parasite seeds per pot had been mixed. The number of emerged *Striga* stems per pot was counted regularly until the termination of trials between 125 and 135 days after planting. In 1994, nine rice lines which had exhibited various levels of parasite susceptibility in pot trials were evaluated at two naturally infested field sites in Cote d'Ivoire. The site at Kouto (9^0 53' N, 6^0 25' W) was infested by *S. aspera* while the field at Dokaha (9^0 24' N, 5^0 41' W) was infested by *S. hermonthica*. The trials comprised eight complete randomised blocks at each site with plots of the susceptible cultivar IAC165 arranged between every second plot of other cultivars under test. Each plot consisted of three 5 m rows spaced 25 cm apart. *Striga* counts were made between the outside rows, excluding 50 cm at each end of a plot. Rice biomass (dry weight) was determined following the final count from a 4 m section of the central row of each plot. Data were analysed by ANOVA, following square root transformation (x+1 for data sets containing zeros) in the case of pot trials, lines on which no *Striga* emerged were excluded from the analysis.

Results and Discussion

In initial pot screening trials (data not presented) *S. aspera*, collected from Kouto, Cote d'Ivoire, did not develop on 18 of the 40 accessions of *O. glaberrima* from west Africa which were tested. This species of *Striga* emerged on all 80 accessions of *O. sativa* evaluated, including landraces from west Africa, cultivars and improved lines from the WARDA breeding programme. Susceptibility ranged from more than 20 parasite stems per rice plant to 2-3 stems for the most resistant IR49255-BB-5-2 and

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THE SELECTION OF RESISTANCE TO *Striga* SPECIES IN UPLAND RICE

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ABSTRACT

Repeated pot trials confirmed complete resistance of *Oryza glaberrima* lines ACC102196, Makassa and IG10 to samples of *Striga aspera* and *S. hermonthica* from Cote d'Ivoire. *O. sativa* lines IR49255-BB-5-2 and IR47255-BB-5-4 also exhibited complete resistance to *S. hermonthica*, and limited susceptibility to *S. aspera*, supporting 2-3 emerged parasite stems per rice plant compared to over 20 on susceptible cultivars which are widely grown in *Striga* infested areas of Cote d'Ivoire. IR49255-BB-5-2 was also shown to be resistant to a sample of *S. asiatica* collected from Zanzibar. Lines shown to be resistant in pot culture were evaluated in the field at *S. aspera* and *S. hermonthica* infested sites in northern Cote d'Ivoire. Although all selected lines supported the development of both *Striga* species, emergence of IR49255-BB-5-2, ACC102196, Makassa and IG10 was delayed and total number of parasite stems was less than that observed on a susceptible cultivar. Despite parasite attack these lines grew vigorously and appear to have the potential to allow increased rice productivity at *Striga* infested sites. *Striga* resistance of an *O. glaberrima* parent, seen in pot culture, was not expressed in the hybrids resulting from a cross with a susceptible *O. sativa* line.

Additional key words: *Striga aspera, S. asiatica, S. hermonthica, Oryza glaberrima, O. sativa.*

Table 1

ADDITIVE MAIN EFFECTS AND MULTIPLICATIVE INTERACTION ANALYSIS OF VARIANCE FOR GRAIN YIELD (KG/HA) AND THE NUMBER OF *OROBANCHE*/PLOT INCLUDING THE FIRST THREE AND TWO INTERACTION PRINCIPAL COMPONENT ANALYSIS (IPCA) AXES, RESPECTIVELY

Source of variation		GRAIN YIELD		NUMBER OF BROOM./PLOT	
	df	M.S.[a]	R^{2b}	M.S.[c]	R^{2b}
Total	747	7.11		9.43	
Treatments	186	20.64	72.3	21.68	57.2
Environment (E)	16	187.40 ***	56.4	138.21 ***	31.3
Replicates within E	51	5.94	5.7	18.27	13.2
Genotype (G)	10	12.03 **	2.3	47.82 ***	6.8
G x E	160	4.50 ***	13.6	8.39 ***	19.1
IPCA 1	25	16.59 ***	57.6	45.82 ***	85.3
IPCA 2	23	4.35 **	13.6	5.08 NS	8.7
IPCA 3	21	3.08 NS	9.0	-.-	-.-
Residual	91	1.54 NS	19.8	0.72 NS[d]	6.0
Error	510	2.29	22.0	4.09	29.6

[a] *Mean Square x 10^5*

[b] *Fraction of sum of squares associated to each term or interaction.*

[c] *Mean Square x 10^4*

[d] *df=112.*

*Hypothesis constructed based on a mixed model, the significance of the AMMI models based on postdiction. NS, *, **, ***: P>0.05; 0.05>P>0.01; 0.01>P>0.001; 0.001>P>0.000, respectively.*

Table 2

AVERAGE RMS PD (KG/HA AND NUMBER OF *OROBANCHE*/PLOT FOR SEVEN MODELS BASED ON 25 RANDOMIZATIONS. DATA ARE BASED ON 11 FABA BEAN CULTIVARS GROWN IN 17 ENVIRONMENTS IN SPAIN

Model	RMS PD	
	Grain yield	Number of broomrapes/plot
AMMI0	430.15	213.43
AMMI1	416.69	187.46
AMMI2	442.79	223.34
AMMI3	458.85	226.66
AMMI4	471.49	228.33
AMMI5	484.62	228.40
DATA	493.56	229.65

GxE interaction were highly significant and accounted for 31, 7 and 19% of the total variation (Table 1).

Both postdictive and predictive assessment selected AMMI1 as the best model (Table 1 and 2). A biplot of the AMMI1 model is given in Fig. 1,b. Four groupings of the genotypes are suggested by Fig. 1,b:

Group 1 includes the broomrape resistant genotypes 2, 8, and 11. They show a similar mean of the number of *Orobanche*/plot and a homogeneous, large positive interaction.

Group 2 includes genotypes 4,9, and 10. The first two are experimental genotypes and 10 is a commercial cultivar which is tolerant although not resistant to *Orobanche*. They show a similar mean near the grand mean but their interactions with environment differ. The interaction PCA1 score for genotypes 9 and 10 are positive and moderate; for genotype 4 is close to zero, and it is therefore the most stable genotype.

Group 3 includes genotypes 1, 3, 7 (commercial cultivars), and genotype 5 (an experimental genotype). They show high mean (above the grand mean for the number of *Orobanche*/plot) and a similar negative interaction PCA1 scores.

Group 4 includes Genotype 6, a susceptible commercial variety. It shows the highest mean for the number of *Orobanche*/plot and its interaction score is the largest negative.

Most of the environment IPCA1 scores are near zero except environments 7, 9, and 16 which show positive environment IPCA1 scores (Fig. 1,b).

Discussion

Results obtained with the AMMI analysis indicated that cultivars "BROCAL", NV3, NV2, "AMCOR", NV5 and "ALAMEDA" are the most yield stable because their IPCA1 scores are near zero (Fig. 1,a).

Concerning the number of *Orobanche*/plot the AMMI analysis indicated that cultivars VF 1071, L2, NV2, L1 and ALAMEDA showed adaptation to most environments, since most of the environments had negative IPCA1 scores while these cultivars had the largest positive IPCA1 scores. In this case a large genotypic IPCA1 score reflects more specific adaptation to environments with IPCA1 scores of the opposite sign.

L1, L2 and VF1071 were by far the most broomrape resistant materials and were as productive as well established cultivars, but their yields showed poor stability among environments. More breeding effort is needed in these three experimental genotypes to improve the yield stability; otherwise, they would only be sown in heavily infected plots, but many farmers would be reluctant to use them in other kind of environments.

Two cultivars, "BROCAL" and "AMCOR", and one experimental genotype, NV2, proved to be the most widely adapted for yield in all environments despite the high number of *Orobanche*/plot recorded in their plots. High density of *Orobanche* is not favourable as it would increase the broomrape seed reserve in the soil. Genotypes from these two groups are valuable for breeding faba beans combining high yield and broomrape resistance.

Our results show that the resistant genotypes and/or cultivars behave better than the susceptible ones in conditions of heavy infestation but these resistant genotypes are not yet as stable as the best yielders even if the latter are susceptible. Thus, it seems necessary to perform one or two more cycles of recurrent selection after crossing the best and most stable yielders with the most resistant genotypes. At the present time, the use of a resistant cultivar has to be recommended to farmers if fields are infested. If they are not, the use of good and stable although susceptible yielders (such as "BROCAL") is the best strategy. If the infestation level is unknown, the farmer can sow "Alameda", or any other highly tolerant and productive cultivar. In this way, the crop can be assured and the level of infestation can be observed.

Both postdictive and predictive assessments were used to analyze the GxE interaction (Gauch and Zobel, 1988). In the postdictive assessments, those IPCAs which were not significant were pooled into the residual term. In the predictive asessment, two random replications for each GE combination were used for construction of the model and the other two replications were reserved as validation observations. The Root Mean Square Predictive Difference (RMS PD) was used as the criterion for predictive success; the RMS PD is the square root of the squared difference between the predicted values and validation observations, summed over all genotypes and environments and divided by the number of validation observations (Nachit *et al.* 1992). A small value of RMS PD indicates good predictive success. The average RMS PD value of 25 validation runs was used.

When one IPCA axis accounts for most GxE, a related graphical aid in interpreting the GxE interaction effects is the biplot suggested by Zobel et al. (1988) (Fig. 1,a,b). Genotypes and environments are plotted on the same diagram, facilitating inference about specific interactions of individual genotypes and environments by using the sign and magnitude of IPCA1 values.

Results

AMMI for grain yield

Genotype and environment main effects and their interaction were highly significant (0.001>P>0.0001) (Table 1), suggesting a broad range of genotypic diversity and environmental variation. Based on postdiction, the AMMI2 model for this study was statistically significant. However, in the predictive sense, the AMMI1 model was superior, showing the least deviation from validation data, based on 25 randomizations (RMS PD of 416.69 kg/ha, Table 2).

The biplot shown in Figure 1,a, simultaneously summarizes information on genotypic and environmental main effects and interactions (IPCA1) as defined by AMMI1. Displacement along the abscissa reflected differences in main effects, whereas displacement along the ordinate illustrated differences in interaction effects. Genotypes with IPCA1 values close to zero show wider adaptation to the tested environments. A large genotypic IPCA1 values reflects more specific adaptation to environments with IPCA1 values of the same sign.

Three groupings of genotypes are evident from Fig. 1,a:

Group 1 includes Genotypes 2, 8, and 11, that is, *Orobanche* resistant genotypes. They show a homogeneous mean yield response close to the grand mean and a similar large negative interaction. For these genotypes, the AMMI1 model predicts genotype yields that are close to those of the AMMI0 model in environments with IPCA1 values near zero, larger yields than the AMMI0 model in environments with negative environment IPCA1 values, and smaller yields than the AMMI0 model in environments with positive environment IPCA1 values.

Group 2 consists of Genotypes 1 and 6, two commercial genotypes, susceptible to *Orobanche* Their IPCA1 scores are positive and large while their mean yields differ from each other.

Group 3 consists of Genotypes 3, 4, 5, 7, 9, and 10. It is a very heterogeneous group, consisting of experimental and commercial genotypes. They show a similar mean response above the grand mean. They show the smallest interactions, and are therefore the most stable genotypes.

The environments show much variability in both main effects and interactions, IPCA1 for environments showing no clear patterns (Fig. 1,a).

AMMI for number of *Orobanche*/plot

Additive main effects and multiplicative interaction analysis showed that environments, genotypes and

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STABILITY OF VARIETIES OF *Vicia faba* RESISTANT TO *Orobanche crenata*

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ABSTRACT

This study analyses the genotype-by-environment (GxE) interaction of 11 genotypes of faba bean, grown in 17 environments in Andalucía (Southern Spain). GxE for grain yield and the number of *Orobanche*/plot was examined with the Additive Main Effects and Multiplicative interaction (AMMI) model. Results of the stability analyses indicated that the most stable genotypes for resistance were not the same as those for yield. However three, L1, L2 and VF1071, were by far the most *Orobanche* -resistant materials and were as productive as well-established cultivars, but their yields showed low stability among environments. Progress in selection for both *Orobanche* resistance and yield is hampered by large environmental variation between locations. More breeding effort is needed in these three experimental genotypes in order to improve the yield stability and therefore acceptability to farmers.

Additional key words: AMMI model, Broomrape resistance, Genotype-Environment interaction, *Vicia faba* L., Yield stability.

Table 1

RESPONSE OF SOME *VICIA* SPECIES TO *OROBANCHE CRENATA* INFECTION IN FIELD CONDITION AT CORDOBA

Species	Number of accessions	*Orobanche* shoots per *Vicia* plant	*Orobanche* shoots per plant of the surrounding rows of Prothabon	Shoots plant relative to Prothabon (%)	Range (%)
V. articulata	5	0.38	6.15	6.6	(0-23)
V. benghalensis	5	1.60	4.86	33.2	(0-110)
V. ervilia	4	3.39	5.21	64.5	(11-166)
V. hirsuta	3	0.96	5.22	7.7	(4-10)
V. hybrida	3	2.50	6.64	37.8	(22-55)
V. lutea	3	1.28	7.84	16.0	(7-22)
V. pannonica	2	1.66	5.76	29.0	(23-35)
V. peregrina	4	0.00	5.88	0.0	(0-0)
V. sativa	17	1.82	6.01	30.3	(2-99)
V. villosa	10	1.87	7.84	23.9	(0-49)
V. cracca	1	5.50	4.50	122.0	
V. fulgens	1	1.00	5.71	18.0	
V. hyrcanica	1	4.00	9.94	40.0	
V. monanthos	1	5.50	8.34	66.0	
V. narbonensis	1	6.75	7.51	90.0	
V. palaestina	1	0.75	7.55	10.0	
V. sicula	1	1.50	7.29	21.0	

Table 2

RATES OF INFECTION AND HOST PLANT CONDITION IN THE PURE LINES COLLECTION OF FABA BEAN

Lines	*Orobanche* shoots/ plant	Shoots/ surrounding Prothabon plant	Shoots/ plant (corrected)	Host condition
Prothabon	3,58	3,58	100 a[1]	Green
Alameda	2.10	3.68	60.7 b	Green
L-1	2.25	3.54	59.5 b	Green
Paucijuga	1.75	3.17	51.3 bc	Green
ILB 4349	1.15	3.33	34.4 bcd	Green
ILB 1825	1.13	3.68	33.0 bcd	Green
ILB 4348	0.98	3.57	25.1 de	Green
VF 1071	0.83	3.41	22.5 de	Green
ILB 4993	0.82	3.96	20.6 de	Green
BPL 241	0.71	3.95	17.6 e	Green
ILB 4351	0.43	3.04	17.4 e	Green
L-2	0.46	3.01	14.7 e	Green
BPL 2210	0.53	3.86	13.8 e	Green
ILB 4350	0.46	4.14	12.3 e	Green
ILB 4347	0.32	2.91	10.3 e	Green

1. Means with the same number are not significatively different (test LSD. $P \leq 0.05$).

Table 1

RATES OF INFECTION AND HOST PLANT CONDITION IN THE PURE LINES COLLECTION OF FABA BEAN

Lines	*Orobanche* shoots/ plant	Shoots/ surrounding Prothabon plant	Shoots/ plant (corrected)	Host condition
VF45	7.64	4.96	168.9 a[1]	Green
VF34	7.06	4.78	164.8 a	Green
VF132	4.09	3.50	138.3 ab	Dead with pods
VF131	4.34	4.24	121.3 abc	Dead with pods
VF121	5.03	4.21	119.9 abc	Green
VF47	5.70	5.23	112.0 abcd	Green
VF61	3.51	3.83	101.7 bcde	Dead with pods
VF49	4.45	4.44	101.1 bcde	Green
VF36	3.36	3.89	100.5 bcde	Green
Prothabon	4.52	4.52	100.0 bcde	Green
VF44	2.90	3.42	82.8 bcdef	Dead with pods
VF167	3.34	4.70	79.6 bcdef	Dead
VF43	3.39	4.34	78.9 bcdef	Green
VF59	2.67	4.02	75.4 cdefg	Dead with pods
VF89	3.44	5.33	70.9 cdefg	Dead
VF46	3.17	4.53	65.4 cdefg	Dead with pods
VF181	1.82	3.70	62.0 cdefg	Dead with pods
VF41	3.23	5.26	61.8 cdefg	Green
VF73	2.15	3.88	61.6 cdefg	Dead with pods
VF147	3.15	5.18	59.0 defg	Dead with pods
VF40	2.71	4.39	58.4 defg	Dead
VF2	1.90	3.85	57.0 defg	Dead with pods
VF72	2.25	4.04	53.8 defg	Dead with pods
VF22	2.83	5.75	52.0 defg	Dead with pods
VF39	2.31	4.75	49.3 efg	Green
VF27	2.67	5.69	46.1 cefg	Dead with pods
VF124	1.99	4.74	44.3 efg	Dead with pods
VF64	1.88	4.51	44.1 efg	Dead with pods
VF97	1.79	4.88	38.5 fg	Dead
VF100	1.53	4.56	36.3 fg	Dead with pods
VF164	1.53	4.99	30.6 fg	Dead
VF54	1.13	3.52	30.2 fg	Dead with pods
VF52	1.49	5.80	28.2 fg	Dead
VF35	1.06	4.20	28.1 fg	Dead
VF38	1.36	4.61	27.5 fg	Dead with pods
VF173	1.12	4.49	26.0 fg	Dead
VF119	1.46	5.43	25.4 fg	Dead with pods
VF26	0.72	3.91	18.4 g	Dead
VF51	0.82	4.40	17.2 g	Dead

1. Means with the same number are not significatively different (test LSD. $P \leq 0.05$).

Introduction

Orobanche crenata Forsk. is a very destructive parasitic weed that seriously limits the cultivation of faba bean in Mediterranean countries. The production of resistant cultivars seems to be the most promising and long-term way of control (Cubero and Hernández, 1991). One of the problems in breeding for broomrape resistance is the lack of an effective selection method. Final number of emerged broomrape shoots per plant is the favourite index.

In the present experiment we studied in detail the resistance of a collection of pure lines inbreed at Córdoba, and of a collection of lines with reported resistance.

Material and Methods

Two sets of plant material were studied in field conditions. The first consisted of a collection of 37 pure faba bean lines (VF-numbers, Table 1) inbreed at Córdoba from material collected in China, Egypt, Spain and South America, kindly provided by Prof. A. Martín. The second set consisted of 16 lines selected on the basis of reported resistance, from CIDA collection or kindly provided by ICARDA (BPL-numbers and ILB-numbers, Table 2).

The lines were sown early December 1994 at Córdoba, in a field heavily infested with *O. crenata*, in a randomized block design with three replicates. Each line was sown in a 1 m row with 10 seeds/row. Each test row was surrounded on four sides by rows of the susceptible cultivar Prothabon, which has been used as reference.

No herbicides were applied, so the plots were hand-weeded when necessary. Due to the severe drought at that season in Córdoba, the plots were watered twice in April.

At the end of the growing season (mid-May) the number of faba bean plants and the number of emerged broomrapes per row were counted. Data per line was referred to the mean of its four surrounding Prothabon rows (set at 100%). Data were processed using the Statistic SX program version 4.1. Differences were tested with the ANOVA and LSD tests (P£0.05). General plant condition and pod formation by the faba bean lines were recorded along the growing season.

Results

Final numbers of emerged *Orobanche* shoots varied markedly between lines. In the pure lines this ranking was from very low (17 % of Prothabon) to very high (169 % of Prothabon) (Table 1). Lines VF45 and VF34 were significantly more susceptible than Prothabon, whereas lines VF97, VF100, VF164, VF54, VF52, VF35, VF38, VF173, VF119, VF26 and VF51 displayed significantly less emerged shoots than Prothabon.

The reaction of the lines of the second set (Table 2) is in agreement with their reported resistance elsewhere. Final number of emerged broomrapes in this collection ranged from low (10.3% of Prothabon) to moderately high (60.7% of Prothabon). Although all the lines were significantly more resistant than Prothabon, some lines as BPL 241, ILB 4351, L-2, BPL 2210, ILB 4350 and ILB 4347 were very resistant while others as Alameda and L-1 were just moderately resistant.

The distribution of the broomrape seeds in the field appeared to be fairly uniform as can be seen in the infection of the check Prothabon all around the plot (Tables 1 and 2).

At the time of broomrape counting we could appreciate three different types of host plant reactions. Some lines remained green and vigorous and produced pods (we called them 'green' type). Others were dying or dead plants, but had produced some pods (we called them 'dead with pods' type). Lines from the third group were dead without pods ('dead' type); they died before flowering or their flowers aborted before maturation. All three types of reaction were found within the pure lines collection (Table 1). However,

Table 1

CHARACTERISTICS OF SOME LINE RESTORERS OF FERTILITY

Number of lines	Resistance to *Orobanche* (%)	Resistance to downy mildew (%)	Habitus	Oil content (%)
4508	100	100	+*	45.1
4531	100	80	+	51.3
4552	100	75	+	48.2
252	100	100	+	50.0
255	100	100	+	48.1
258	100	100	+	50.2
281	100	100	+	50.4
258	100	100	+	48.0
263	100	100	+	49.8
264	100	100	+	44.5
269	100	100	+	47.2
271	100	100	+	48.1
283	100	100	+	44.8
254	100	100	+	42.3

*+ mean branched habitus.

Table 2

CHARACTERISTICS OF SOME HYBRIDS

Number of lines	Resistance to *Orobanche* (%)	Resistance to downy mildew (%)	Yield (%) of seeds
2607 x 4531	100	90	99.5
2607 x 4552	100	52	108.0
2607 x 258	100	100	101.4
2607 x 263	100	100	107.9
3064 x 269	100	100	106.8
2607 x 271	100	100	127.5
2607 x 281	100	100	111.8

Results

As the result of the directed selection during the period 1987-1995, the developed line-restorers of fertility had to be resistant to two main pathogens, *Orobanche* and downy mildew. These lines also possessed other valuable characters: higher oil content, good combining ability and branched habitus (Table 1).

In 1994 the best lines were selected as male components of new hybrids. The data in Table 2 present the results of phytopathological evaluation of these hybrids in the greenhouse on the basis of their seed yield. All hybrids possessed full resistance to *Orobanche,* five of them possessed resistance both to *Orobanche* and to downy mildew. The hybrid combination 2607 x 271 was characterized by 27.5% seed yield above the standard, and the combination 2607 x 281 by an addition of 11.8%.

Seeds from hybrids shown in Table 2 were sent to Trace Agricultural Research Institute at Edirne, Turkey and to VanderHave in Spain, where their reaction to local populations of *Orobanche* was evaluated. The results demonstrated that the resistance in our hybrids is also effective against Turkish and Spanish races of *Orobanche*, that are not more virulent than Bulgarian races.

Conclusion

As a result of our purposeful and long-term research new line-restorers of fertility, resistant to *Orobanche* and downy mildew, have been developed. They have been used in new resistant hybrids with high seed yields. It might be possible to grow them in regions with high *Orobanche* infestation in Bulgaria, as well as in Turkey and Spain.

Acknowledgments

We would like to express our thanks to our colleagues A. Aydin from Trace Research Institute at Edirne, Turkey and A. Thompson and E. Vranken from VanderHave for their assistance in testing of the breeding materials for resistance to *Orobanche*.

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V.15

BREEDING OF SUNFLOWER LINE-RESTORERS OF FERTILITY, RESISTANT TO BROOMRAPE, *Orobanche cumana*

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ABSTRACT

Open pollinated sunflower varieties resistant to *Orobanche*, from Russian and Ukrainian breeding, as well as from interspecific crosses and local populations were used as sources for sunflower breeding. They were crossed with our line-restorers with good combining ability, resistant to downy mildew, but susceptible to broomrape. Two breeding methods were used, backcross and new breeding with initial forms. In each generation breeding was done for resistance to broomrape. As a result line-restorers were created, resistant to broomrape and to downy mildew.

Table 3

EVALUATION OF 2 SUNFLOWER HYBRIDS AND THEIR RESPECTIVE PARENTALS WITH E3 OROBANCHE ISOLATE

Line/ Hybrid	EMERGED BROOMRAPE		BROOMRAPE ON ROOT	
	Incidence (%)	Severity	Incidence (%)	Severity
CMS1132	80	6.2	100	19.6
R1818	0	0	0	0
CMS1132 x R1818	90	10.2	100	18.8
CMS106	100	14.6	100	20.4
R1522	30	0.6	55	2.1
CMS106 x R1522	95	11.4	100	20.4

Introduction

Sunflower is affected severly by *Orobanche cernua* Loeft. (sin *O. cumana* Wallr.) in several countries mainly arround Black Sea and in Spain. The control of this parasite plant has been mainly made by the use of resistant cultivars (see review, Cubero, 1991).

In Spain, since the begining of the 90's, the sunflower broomrape situation has drastically changed due to the appearance and dispersion of virulent pathotypes that infested all comercial hybrids that were available at that moment (García-Torres *et al.*, 1993). After 1993, several sunflower hybrids resistant to the new *Orobanche* pathotypes have been developed and comercialized. Most of them carrying the Or_5 gene as the source of resistance.

The goal of this paper is to find out the behaviour of these resistant hybrids with respect to different broomrape isolates collected in Spain during the 1994 and 1995 years.

Material and Methods

Fifteen commercial sunflower hybrids were tested. Two of them (T1, T2) *Orobanche* sensible to the new pathotypes and thirteen (H1 to H13) catalogued as *Orobanche* resistant by the registration official trials carried out by the Instituto Nacional de Semillas y Plantas de Vivero in 1994 and 1995. Six Koipesol lines (CMS1132, CMS1422, R1311, R1522 and R1021) were also included in the test. The line R1021 carries the Or_5 gene of broomrape resistence (Vrânceanu *et al.*, 1980), the other lines have unknown resistance genes.

The following four *Orobanche* isolates were included in the trial:

– C1 isolate. Collected in 1994 on a susceptible oil sunflower hybrid resistance to the *Orobanche* isolates prevalent in Spain up to 1992 in Rozalen del Monte, Cuenca, Central plateau of Spain.

– E1 isolate. Collected in 1994 on a susceptible oil sunflower hybrid resistant to the isolates prevalent in Spain up to 1992 in Ecija, Sevilla, South of Spain.

– E2 and E3 isolates were collected in 1995 in two farms near Ecija, where resistant hybrids to the isolates C1 and E1 were highly broomrape infected.

The experiments were carried out under controled conditions in a greenhouse. The inoculation method was similar to the previously one described by Melero *et al.*, (1989). The scoring of results was performed in 20/25 sunflower plants in maturation state. Only the emergent broomrapes were scored. The severity of infection was obtained dividing the total number of emerged broomrape plants by the total number of sunflower plants studied.

Results and Discussion

The first experiment was carried out to corroborate the resistance of some commercial hybrids and some parental lines with respect to the most prevalent *Orobanche* populations in Spain. For this purpose, the sunflower material was tested with E1 and C1 isolates. With respect to pathogenity, these isolates are representative of the *Orobanche* populations from the South (E1) and Center (C1) of Spain (unpublished data; Melero, com. pers.).

As expected, all the hybrids and lines showed a highly resistant reaction towards E1 (Table 1). With respect to C1, 8 hybrids were highly resistant and the other 5 showed only a moderate resistance (table 1). These results agree with those obtained in the CIDA Trials (Dominguez, pers. com.). In addition, the line CMS 1132 was resistant to E1 and sensible to C1 (table 2). These results indicate that the *Orobanche* populations found in the Central plateau and in the South of Spain show a clear difference in pathogenity. CMS1132 could be used as a differential sunflower line to distinguish both *Orobanche* populations.

V.14

NEW HIGHLY VIRULENT SUNFLOWER BROOMRAPE *(Orobanche cernua Loefl.)* PATHOTYPES IN SPAIN

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ABSTRACT

At the begining of the 90's, the sunflower broomrape problem has dastrically cahnged due to the appearance of virulent pathotypes that infested all commercial hybrids in Spain. After 1993, several sunflower hybrids resistant to new *Orobanche* pathotypes have being developed.

Several resistant sunflower hybrids and parenta lines, under controled conditions, were tested to 4 *Orobanche cernua* isolates. One of these isolates (C1) was collected in Cuenca (Central Spain) over sensible sunflower; the other three were collected in Ecija (South Spain), one of these over sensible sunflower (E1) and the other two over new resistant sunflower hybrids (E2, E3).

All the hybrids and lines tested showed a resistant reaction towards E1. Most of them showed a resistant reaction towards C1 and all of them were sensible towards E2 and E3. These results suggest the presence of at least three pathotype groups of *Orobanche cernua* that attack the oil sunflower in Spain.

Furthermore, when using the E3 isolates, homozygous resistant lines showed lower percentage of *Orobanche* attack than heterozygous hybrids. These results suggest that resistance to *Orobanche* may have additive inheritance in some cases.

Table 1

REACTION OF A SET OF SUNFLOWER DIFFERENTIAL HOSTS TO THE BROOMRAPE COMPLEX OF RACES IN TWO LOCATIONS OF SOUTHERN SPAIN (ANDALUSIA)

Differential	Number of sunflower plants observed		Frequency of *Orobanche* attack (%)		Average number of *Orobanche* stalks per plant (Severity)	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
AD-66	61	32	100	100	19.70	19.30
Kruglik-41	72	24	100	100	9.70	21.00
Vnimmk-8931	81	24	85	100	10.40	24.80
Record	52	26	50	100	5.01	8.23
S-1358	76	30	22	100	1.34	10.20
P-1380-2	50	20	0	0	0.00	0.00
l.s.d. (5%)					9.40	4.60

Table 2

THE ARRAY OF PHYSIOLOGICAL RACES OF THE SUNFLOWER BROOMRAPE[a] AND THE PROPORTION OF EACH RACE IN TWO LOCATIONS OF ANDALUSIA

Differential host	Broomrape races					Resistance reactions	Resistance genes
	A	B	C	D	E		
AD-66	S[b]	S	S	S	S	R0	-
Kruglik-41	R	S	S	S	S	R1	Or1
Vnimmk-8931	R	R	S	S	S	R2	Or2
Record	R	R	R	S	S	R3	Or3
S-1358	R	R	R	R	S	R4	Or4
P-1380-2	R	R	R	R	R	R5	Or5
Proportion of races (%)							
Experiment 1 (La Carlota)	47.2	0	27.3	18.6	6.8		
Experiment 2 (Ecija)	0	0	58.9	0	41.1		

a. According to Vranceanu et al. (1980).

b. S = susceptible, R = resistant.

When race group (n-1) severity is lower than race group (n) severity, the presence of (n-1) race can not be detected, thus, the value to use for calculating the proportion is the race group severity corresponding to (n-2) race group, and so on.

Results and Discussion

The values of the incidence (frequency) and severity of *Orobanche* infections for each differential in each experiment are given in Table 1. The incidence in experiment 2 (Ecija), was 100 % for all the differentials, except P-1380-2 which was resistant while differentials showed different degrees of incidence in experiment 1 (La Carlota). According to the data of severity, it seems that all races, except race B, are present in experiment 1. The presence of races D and E, already detected in Spain by Refoyo and Fernandez (1994), is confirmed now, therefore suggesting the inadequacy of growing cultivars that do not carry the Or_5 gene. The situation is even more extreme in experiment 2 (Ecija), where only races C and E have been detected, both in high proportions. The fact that race E is in such a high percentage (41.1 %) and that the majority of cultivars cropped in this area are resistant to this race, therefore carrying the Or_5 gene, might be of a certain danger, since the possibility of mutation or recombination giving raise to the selection of a new and more virulent race is higher than in the fields surrounding experiment 1.

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V.13

VIRULENCE GROUPS OF *Orobanche cernua* IN ANDALUSIA (SOUTHERN SPAIN)

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ABSTRACT

Orobanche cernua Loefl. has proven to be an extremely dangerous parasite of sunflower in Southern Spain (Andalusia) where important damages caused by this parasitic angiosperm have been reported in the nineties. In order to know the current racial composition of broomrape populations, two field nurseries were planted in places where heavy infections of broomrape had been reported previously in the provinces of Seville and Cordoba. Following the methodology defined by Vranceanu et al. (1980), the proportion of each physiological race to the total broomrape population was calculated in accordance with the average intensity of the attack on a set of sunflower differentials. Five types of resistance, which correspond to the five virulence groups identified in the parasite, were established. The occurrence of individuals from the majority of the five different virulence groups was assessed, including those of the most evolved race complex, E. Even so, P-1380-2, a differential line carrying the Or_5 gene, showed to be resistant to all race complexes present in the experimental fields.

Table 2

REACTIONS OF 16 ANNUAL *HELIANTHUS* SPECIES TO *OROBANCHE CERNUA*[a]

PI No.	Species	2n	Incidence (%)	Degree of attack[b]
468415	*H. agrestis*	34	0.0	0.0
413017	*H. annuus*	34	100.0	12.5
468638	*H. anomalus*	34	0.0	0.0
468651	*H. argophyllus*	34	100.0	4.0
468660	*H. bolanderi*	34	100.0	14.0
	H. debilis			
435667	*ssp. cucumerifolius*	34	80.0	3.0
468674	*ssp. silvestris*	34	80.0	8.0
468668	*ssp. tardiflorus*	34	71.0	2.0
435664	*H. exilis*	34	0.0	0.0
	H. niveus			
435772	*ssp. canescens*	34	100.0	3.2
468791	*ssp. niveus*	34	100.0	2.2
	H. petiolaris			
468810	*ssp. fallax*	34	80.0	3.0
503232	*ssp. petiolaris*	34	65.0	3.0
	H. praecox			
435884	*ssp. hirsutus*	34	65.0	1.2
435847	*ssp. praecox*	34	65.0	2.0
468860	*ssp. ruyonii*	34	65.0	2.0

a. Sunflower seedlings were potted in sterile soil infected with seeds (200 mg/kg of soil) of population SE194 of O. cernua.
b. Average number of emerged broomrapes per sunflower plant.

Table 3

REACTIONS OF INTERSPECIFIC HYBRIDS OF *HELIANTHUS EXILIS* WITH CULTIVATED SUNFLOWER TO *OROBANCHE CERNUA*

Croses	PLANTS (No.)		
	Total No. of plants	Resistant	Susceptible
H. exilis 1 x S59	3	3	0
H. exilis 4 x S59	2	2	0
H. exilis 3 x S59	1	1	0
H. exilis 1 x P21	22	19	3
H. exilis 3 x P21	25	25	0
H. exilis 5 x P21	1	1	0
H. exilis 3 x HA89	1	1	0
H. exilis 1 x R5	1	1	0

V.12

EVALUATION OF WILD *Helianthus* GERMPLASM FOR RESISTANCE TO HIGHLY VIRULENT RACES OF *Orobanche cernua* LOEFL. AND TRANSFER TO CULTIVATED SUNFLOWER

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ABSTRACT

Twenty six different wild perennial species of sunflower and 16 wild annual species were evaluated for susceptibility to a virulent population of *Orobanche cernua* under artificial conditions. Most perennial wild species except *H. gracilentus* and *H. nuttallii* were immune to *Orobanche*. Wild annual species were susceptible except *H. agrestis*. *H. anomalus* and *H. exilis*, which showed a resistant reaction. On the basis of these results, we made crosses between *H. exilis*, a resistant annual sunflower species, and susceptible cultivated lines, as well as with the latter and a resistant line carrying the resistance gene Or_5, which is used in the production of hybrids in Spain. Current work is aimed to determine which resistance genes are present in these species.

Key words: Parasitic weeds, disease resistance, interspecific hybrids.

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V.11

VARIATION IN VETCH (*Vicia* spp.) RESPONSE TO *Orobanche aegyptiaca* PERS.

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ABSTRACT

Experiments conducted in pots containing soil inoculated with *Orobanche aegyptiaca* seeds demonstrated that purple vetch (*Vicia atropurpurea*) of the genotypes Popany and Sadot are resistant (R) to the parasite, whereas common vetch (*Vicia sativa*) genotypes 'Yovel' and 473-A are susceptible (S). Purple vetch genotypes grown in-vitro stimulated a significantly higher rate of parasite seed germination than that stimulated by common vetch genotypes. *O. aegyptiaca* attachments failed to develop into advanced parasite stages on the R host roots, whereas many parasites matured on the S host roots. Microscopic studies revealed that when inoculated with *O. aegyptiaca*, the R vetch genotypes develop necrotic lesions surrounding the contact points of the parasite's radicle. Further development of the parasite is stopped either in the root cortex or when reaching the vascular cylinder. A reddish-brown secretion was observed at the host-parasite interface filling apoplastic spaces including the cavities of R host vessel elements.

Additional key words: forage-crop, holoparasite, legume, Mediterranean, *Orobanche crenata*.

Table 1

RANGE OF DISTRIBUTION OF TOBACCO GERMPLASM TO *OROBANCHE* INCIDENCE IN DIFFERENT YEARS

Year	Plants infested (%)	Spikes/plant	Wt. of spikes /plant (g)
1992-93	12.5 - 87.5 (69.7)	1.2 - 20.5 (7.6)	3.0 - 116.0 (56.2)
1993-94	13.0 - 87.5 (53.1)	0.2 - 8.8 (3.0)	1.9 - 90.0 (27.1)
1994-95	34.2 - 100.0 (76.7)	2.0 - 27.4 (7.5)	12.2 - 116.7 (42.8)

Figures in parenthesis indicate average values.

Table 2

DISTRIBUTION OF REACTION OF TOBACCO GERMPLASM TO *OROBANCHE CERNUA*

Reaction	Plants infested (%)				Spikes/plant				Wt. of spikes/plant (g)			
	Number of accessions				Number of accessions				Number of accessions			
	scoring	92-93	93-94	94-95	scoring	92-93	93-94	94-95	scoring	92-93	93-94	94-95
T/R	<10	0	0	0	<1	0	0	0	<10	1	7	0
L	11-25	1	3	0	1.1-3.0	4	32	2	11-25	5	23	11
M	26-50	5	26	1	3.1-6.0	11	21	24	26-50	18	26	35
H	51-75	32	27	18	6.1-11.0	37	4	26	51-75	23	2	11
VH	>75	22	4	41	>11.1	8	0	8	>75	13	2	3

T/R = Tolerant/resistant, L = Low, M = Medium, H = High, VH = Very high.

Table 3

REACTION OF SELECTED ACCESSIONS TOWARDS *OROBANCHE* IN DIFFERENT YEARS

Acession	INCIDENCE OF *OROBANCHE*								
	1992-93			1993-94			1994-95		
	PI (%)	S/P	Wt (g)	PI (%)	S/P	Wt (g)	PI (%)	S/P	Wt (g)
Bright Caspalia	12.5	1.2	3.0	13.9	0.3	4.9	61.1	2.9	16.2
Bel 61-11	37.5	3.1	24.0	30.0	1.3	14.5	62.5	3.4	24.4
Coker 254	60.5	4.4	31.0	66.9	4.6	35.4	58.7	1.9	8.9

PI(%) = Percent plants infested; S/P = Spikes per plant (average); Wt(g) = Green weight of spikes per plant (average).

years (Table 3). However, higher percentage of infested plants was observed during 1994-95 in case of Bright Caspalia and Bel 61-11, in comparison to earlier years. Coker 254, though, showed higher percentage of infested plants, but had less *Orobanche* spikes per plant in all the three years. With regard to green weight of spikes per plant, Bright Caspalia recorded lowest in 1992-93 and 1993-94 seasons and Coker 254 recorded lowest in 1994-95 season.

Discussion

The results indicated wide variability among the accessions tested with respect to percentage of *Orobanche* infested plants, number of *Orobanche* spikes per plant and green weight of *Orobanche* spikes per plant. Only three accessions namely Bright Caspalia, Bel 61-11 and Coker 254 showed promise with lower values. Percentage infested plants was higher in these three accessions only during 1994-95 season compared to 1992-93 and 1993-94, but the infested plants were lowest when compared to the other accessions tested during 1994-95. Such reports of inconsistency of tolerance to *Orobanche* over seasons have been cited in literature (e.g. Kabulov and Tashpulatova, 1974). It is proposed to confirm the reaction of these three accessions for two more seasons; these will be utilised in breeding programme for producing FVC tobacco with less susceptibility to *Orobanche*. Similar assessment and breeding programmes were carried out by different teams of Soviet and Bulgarian scientists but so far no variety with a complete *Orobanche* resistance was released for cultivation.

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Introduction

Orobanche is a serious problem in India and several other countries. Trials of controlling this parasite using different biological means like fungi and insects at field level are not successful. Screening germplasm or breeding varieties for resistance will have better prospects for controlling this problem like many other soil borne plant pathogens. Crops having resistance to *Orobanche* have been reported but in tobacco, such studies are not successful. In tobacco, plants/varieties with lower incidence of *Orobanche* infestation have been isolated or bred from different crosses in the erstwhile Soviet Union and Bulgaria, but no cultivar has been released with resistance to *Orobanche*. The plants showing no/less incidence of *Orobanche* one year failed to show similar trend during subsequent years. With this background information, attempts were initiated to screen the large number of tobacco germplasm available with the Central Tobacco Research Institute (CTRI), Rajahmundry, to assesss the reaction of different accessions towards *O. cernua* and identify resistant ones, if any, for future breeding work.

Materials and Methods

Every year, sixty accessions were selected in a serial order from the Institute's germplasm collection. Sixty day old seedlings were planted in two rows of 20 plants each in *Orobanche* infested soil and normal cultural and plant protection practices were followed. Emergence of *Orobanche* spikes usually initiated 40 to 45 days after planting and maximum emergence was observed around 60 to 65 days after planting. Incidence of *Orobanche cernua* Loefl. was recorded on individual plants, as number of *Orobanche* spikes emerged around each tobacco plant and total green weight of uprooted spikes. Such observations were recorded at 70 and 100 days after planting. The percentage of infested plants, average number of *Orobanche* spikes per plant and average green weight of spikes per plant in each accession were calculated. Accessions showing lowest of one or more of the above three parameters were retested during subsequent year(s) on a larger population, i.e. 5 rows of 20 plants each.

Results

A total of 180 tobacco germplasm accessions were assessed for their reaction to *Orobanche* under natural field conditions during the last three years. In general, the accessions varied widely in all the three indices. *Orobanche* incidence was more during 1992-93 and 1994-95 than 1993-94, with regard to percentage of plants infested (Table 1). Very few plants remained uninfested during these two years and more accessions showed high to very high percentage of incidence. During 1993-94, most accessions showed medium to high incidence (Table 2). However, none of the 180 accessions assessed showed below 10 percent infested plants and only 4 accessions showed medium incidence, i.e. 11-25% infested plants.

Regarding average green weight of *Orobanche* spikes around each tobacco plant, nearly half of the accessions had 26-50 g/plant. 39 accessions had 11-25 g per plant and only 8 accessions had less than 10 g/plant. The remaining 64 accessions had more than 50 g green weight of *Orobanche* spikes, out of which 18 had more than 75 g and three had more than 100 g. Similarly, only three accessions showed less than one shoot per plant on an average. More than half of the total accessions showed light to moderate reaction (38 in the range of 1.1 to 3.0 and 56 in the 3.1 to 6.0 spike per plant). 67 accessions have shown more than 6.1, and 16 more than 11 shoots per plant on average.

Eleven accessions showing lower values of one or more of the *Orobanche* incidence parameters were selected from 1992-93 trial, and re-assessed during 1993-94 and 1994-95. Similarly, accessions showing lower incidence during 1993-94 were also assessed during 1994-95. Bright Caspalia, Bel 61-11 and Coker 254, selected from 1992-93 trial, continued to show lower incidence in the next two

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VARIABILITY IN TOBACCO GERMPLASM TOWARDS *Orobanche* INFECTION

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ABSTRACT

The Central Tobacco Research Institute, Rajahmundry in India has a large germplasm collection of over 1600 accessions including different types of tobacco. Reaction of 180 germplasm accessions was screened for *Orobanche* infection in an infested field. Observations on number of *Orobanche* shoots emerged around each plant, percentage of infected plants, and green weight of shoots per plot were recorded 70 and 100 days after planting. Accessions showing lowest of the above three parameters were retested during next year. 148 accessions showed more than 50% infested plants, while 28 showed 25-50%. Only four showed less than 25%. On an average, three accessions had more than 6 shoots per plant. Among the remaining accessions, 37 had 1.1 to 3.0 shoots/plant and 57 had 3.1 to 6.0 shoots/plant. The average green weight of *Orobanche* spikes per plant is less than 10.0 g/plant i 7 varieties, 10.1 to 25.0 g/plant in 40 varieties and more than 25.0 g/plant in 133 varieties. Out of the selected varieties retested during 1993-94 and 1994-95 seasons, Bright Caspalia, Bel 61-11 and Coker 254 showed promise.

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peroxidase, H_2O_2 and phenolic substrates are present in the cell wall, all components, capable of reactions form a non-specific net work. By locally activating the peroxidase and increasing secretion of the phenols capable of reaction, the cell wall is strengthened much quicker in a distinct area than by a lignification and suberinisation respectively.

However, extra cellular peroxidase could also be involved in the production of toxic compounds, possibly participating in defence reactions (Förster et al. 1995) or in the metabolism of H_2O_2 produced via conversion of active oxygen species, which are also discussed as being involved in plant defence reactions (Doke 1983, Vera-Estrella et al. 1992).

In summary we can ascertain that the different reactions of tomato plants, e.g. their susceptibility or resistance to *Cuscuta*, depending on their age during infection, is an impressive illustration of the competitive character of the two processes: the attack of the parasite and the defence reaction of the infected plant.

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Introduction

Concerning the parasitism of *Cuscuta* species on tomato, rather contradictory observations exist. According to Ashton and Santanana (1976) and Hutchinson (1977) *Cuscuta campestris* is considered as a weed in the cultivation of tomato. In contrast, Tsivion (1979) and Nemli (1987) described tomato as being resistant to the same *Cuscuta* species and Ihl et al. (1988) to *Cuscuta reflexa*. According to Gaertner (1950) *C. campestris* was found to be thriving or dying on tomato plants and also in the studies of Al-Menoufi and Ashton (1991) some wild species of tomato proved to be susceptible to *C. campestris* and some to be more or less resistant. Furthermore, *Lycopersicon esculentum* proved to be both susceptible and resistant to *C. planiflora*, depending on the host on which the dodder seed ripened (Mamluk and Weltzien, 1978).

The resistance of tomato towards infection by *Cuscuta*, if it occurred, is due to a hypersensitive reaction of the external cell layers of the stem or petiole of tomato after contact with the adhesive secretory epithelium of the parasites prehaustoria . As a result of this reaction a layer of dead tomato cells is formed between host and parasite, preventing the intrusion of the initial haustoria. The hypersensitive reaction is restricted to the external cell layers of tomato plants, because when this layer was partially removed, functional haustoria were able to form on this specific area (Ihl et al. 1988). The results presented provide some information about physiological factors possibly involved in the hypersensitive reaction of tomato plants.

Material and Methods

Plant Material

Stock cultures of *Cuscuta reflexa* ROXB., *C. odorata* RUIZ et PAV., *C. platyloba* PROGEL. and *C. japonica* CHOISY , all parasitizing on *Pelargonium zonale* L., were cultivated under greenhouse conditions with 16 h light and 8 h of darkness at 18 - 24 ^{0}C. These cultures served as sources for vegetative *Cuscuta* shoots used for the infection experiments. *Cuscuta europaea L.* was collected from a natural location on *Humulus lupulus L.* as the host.

Most of the experiments with tomato plants were performed under the greenhouse conditions, mentioned above, too. Only infection experiments using different cultivars of tomato were done under field conditions. The seeds of wild species of *Lycopersicon* were generously donated to the department by Dr. Crick, Tomato Genetic Stock Centre, University of California, Davies.

For infection, isolated stems of the parasites were transferred to tomato stems, using tomato seedlings for infection exclusively on their hypocotyl region. The coiling was also registred as the further development or gradually dying of the parasites. Samples of infected tomato hypocotyls and non-infected ones, respectively were taken at various time intervals for determination of peroxidase activity.

Determination of peroxidase activity

Plant material was homogenised using a cold Tris-phosphate buffer (260 mM, pH 6.9), centrifuged at 20000 x g for 20 min and the supernatant was assayed for cytosolic peroxidase activity (cyp). For the determination of both, ionically and covallently cell-wall bound peroxidase activities the insoluble residue was washed twice with cold 1 % Triton X-100 and after than sometimes with water to remove the detergent .The washed insoluble material, retained by this procedure, was suspended and stirred with 1 M NaCl over a period of 12-14 hours, centrifuged at 20000 x g for 20 min and the supernatant was assayed for ionically bound cell-wall peroxidase activity (iobp). The insoluble residue was washed with water at 4^{0} C (five times) to remove NaCl, centrifuged for 20 min at 20000 x g and after that incubated for hours at 24^{0}C with a

Introduction

Until now the bases of resistance of crop plants to parasitic weeds have not been extensively investigated. This aspect is however an important one because of its possible applications in agriculture. Among parasitic weeds *Cuscuta* (dodder) is a very-appropriate.model for resistance studies and the main stages of development of *haustoria* are known (Dörr, 1987). Previous research has shown that different mechanisms are involved in resistance to *Cuscuta*, depending on the host plants and *Cuscuta* species considered. The resistance of tomato to *C. campestris* results from an hypersensitive reaction, located in the superficial tissues of the host stem, occurring early after *Cuscuta* shoot coiling and impeding penetration of haustoria (Al Menoufli and Ashton, 1991).The resistance of cotton to *C. lupuliformis* occurs later, when haustoria begin to invade cortical tissues of the host stem. Rapid division and differentiation of host cortical cells result in the formation of an isolation layer, made of suberized cells, around the penetrating haustoria (Capdepon, *et al.* 1985).

The aim of the present work was to compare the responses, at the cellular and biochemical levels, of two legume species, a sensitive one (*Phaseolus aureus*) and a resistant one (*Phaseolus vulgaris*), to the same *Cuscuta* species (*C. reflexa*).

Materials and Methods

Plant Material

Two species of *Phaseolus* (*P. aureus* and *P. vulgaris*) were previously shown to be respec-tively sensitive and resistant to *C. reflexa* (Arnaud, 1994). Host legumes were cultivated alone, under controlled conditions, for 3 weeks before infestation. The apical part of *C. reflexa* shoots (800 ± 10 mg, F.W.), was allowed to coil around the host stem. Coiling was considered as the beginning of infestation. Other host plants were kept uninfested and used as controls. Samples of infested and uninfested hosts were harvested at different times from infestation.

2. Analytical Methods

Cuscuta growth measurement: Following attachment of *Cuscuta* stem tips to the legumes, stem elongation and changes in fresh and dry weight were mesured.

Histological techniques: Transversal and longitudinal sections (2 m) from samples embedded in EPON were used to compare the development of haustoria into the two legume species (ARNAUD, 1994).

Histochemical and histofluorescence techniques: 20 m thick sections were cut in fresh material. Polyphenolic compounds were located by epifluroescence, (Salle et al. 1991). Cell wall-bound peroxidases were located using syringaldazine, (Goldberg et al. 1983).

Measurement of Peroxidases (POX) and Phenylalanine ammonia Lyase (PAL): The enzymes assayed are necessarily present both in the hosts and the parasite. To assess the possible changes in the activity of these enzymes in response to parasitism, the samples to be analyzed were carefully selected (Fig. 1).

Measurements of PAL activity were performed according to Rathmell (1973).

POX was assayed using the method described by Ridge and Osborne (1970) which allows separation of the whole enzymatic activity into 3 fractions : soluble (S. POX), ionic wall-bound (I. POX) and covalent wall-bound (C. POX) peroxidases.

Ethylene production measurement: Ethylene production was measured separately on stem segments of legumes and in *Cuscuta* (see result section for details). Production of wound ethylene was prevented by applying a 1 mM $CoCl_2$ solution to the cut parts of stem segments. After incubation

Similar anatomical processes excepting the enlargement of tomato cells (highly specific for resistance towards *Cuscuta*) were observed after artificial wounding of tomato stems. Starting approximately at the 3rd day following mechanical injury, cells of the collenchyma and parenchyma adjacent to the wounding site formed a wound periderm through tangential cell divisions. Cell walls of this secondary boundary tissue were suberized and, in the outermost tissue layers, lignified. The respective cell walls showed light-blue autofluorescence as observed for the scalariform tissue involved in the defence reaction towards *Cuscuta* (Sahm *et al.*, 1995).

Phytochemical and enzymatic analysis

Analysis of methanolic extracts from control tomato samples, as well as from tissue segments of internodes neighbouring the treated internode (NI) by HPLC yielded a simple pattern of soluble phenylpropanoids dominated by the flavonol glycoside rutin (average conc. of 0.112 nmol/mg fw) and the caffeic acid depside chlorogenic acid (av. conc. of 0.067 nmol/mg fw). In addition, a still unidentified hydroxycinnamic acid derivative (UV absorption 284 nm and 318 nm) was found only in infection sites (IIT).

During *Cuscuta* attack, starting at the 3rd day of infection, chlorogenic acid and the unidentified hydroxycinnamic acid derivative showed a clear increase in concentration in the infection sites (IIT). The maximum was measured at the 6th day following infection (1.400 nmol/mg fw for chlorogenic acid and 0.151 nmol/mg fw for the unidentified compound; Fig. 1 and 3). Concentrations of rutin remained laargly unaffected.

Measurement of PAL activity, one of the key enzymes of phenylpropanoid metabolism, revealed no significant increase (16 pkat/mg fw)in infected tomato stem segments, whereas a clear stimulation of the activity of total peroxidases by *Cuscuta* infection was evident (maximum between the 4-6th day of infection: 535% of NI; Fig. 4).

Artificial wounding of tomato stems (WIT) resulted in similar increases in the accumulation of chlorogenic acid and of the unidentified hydroxycinnamic acid derivative, as observed in tissue infected by the parasite. The highest concentration of chlorogenic acid in the mechanically wounded tissue (WIT) was detected at the 4th day following injury (0.743 nmol/mg fw; Fig. 2) and between the 4th and 6th day for the unidentified compound (0.073 nmol/mg fw; Fig 3).

The phytochemical reaction of tomato to infection by *C. reflexa* or to mechanical injury was locally restricted, which was confirmed by analysis of tissue samples from the treated internode (IIN and WIN) or from the following internode towards the apex (controls; NI). When compared to treated tissue (IIT and WIT), only a weak response of secondary metabolism was observed in IIN and WIN. Chlorogenic acid showed an insignificant increase in IIN (maximum; 0.252 nmol/mg fw; Fig. 1), whereas the unidentified compound could not be detected. Peroxidase activity of 0.677E/min/g protein was measured in IIN, which is half as much as that from infected tissue (IIT:1.117E/min/g protein; Fig. 4). Untreated neighbouring internodes (NI) were completely unaffected by the parasitic attack (Sahm *et al.*, 1995).

Discussion

In contrast to other incompatible hosts such as cotton and *Hibiscus rosa-sinensis* (Cardenon *et al.*, 1985; Schlenzka, 1992), that interfere with the already penetrated differentiated haustorium of *Cuscuta*, the anatomical defence reaction of tomato towards *Cuscuta* prevent haustorial development at earlier stages of infection. The contact with the adhesive secretory epithelium of prehaustoria causes an intensive cell enlargement in the affected tomato tissue, followed by necrosis of these cells and formation of a periderm-like tissue with suberized and lignified cells adjacent to the

supernatants dried under a nitrogen stream and dissolved in 100l methanol for HPLC analysis (Sahm *et al.*, 1995).

For quantitative determination, kaempferol was used as internal standard. The content of the unidentified hydroxycinnamic acid derivative was calculated as kaempferol equivalents, whereas the contents of chlorogenic acid and rutin were corrected according to their specific extinctions.

Protein extraction

Fresh tissue segments were crushed in the presence of liquid N_2 in a test tube containing 250l buffer (100mM sodium borate, 10mM mercaptoethanol, pH 8.8 for PAL extraction; 100mM sodium borate, pH 7.5 for peroxidase extraction) per 100mg fw and Polyclat AT (10mg/100mg fw). After removal of the tissue residues by centrifugation (10.000rpm, 2min) the supernatants were stored at -80C. Protein was determined according to Bradford (1976).

Determination of PAL and peroxidase activity

The assay for determination of the activity of phenylalanine ammonia-lyase (PAL) was modified from Koukol and Conn (1961). Assay mixtures containing 90l crude protein extract and 10l L-phenylalanine (20mM) were incubated for 40min at 50C. The formation of E-cinnamic acid was measured by HPLC (Sahm *et al.*, 1995).

Peroxidase activity was measured as ferulic acid dimerization capacity according to Pfanz and Oppmann (unpublished data) modified from Machackova et al. (1975). 10mM H_2O_2 and 5mM ferulic acid were added to 1ml of 100mM Na_2HPO_4 buffer (pH 7.8). After starting the assay reaction by addition of 5l of the respective crude protein extract, absorbance changes due to production of diferulic acid were detected at 400nm.

Results

Morphological and anatomical characterization of the defence reaction

The defence reactions of tomato against *Cuscuta* ssp. were rather similar for all *Lycopersicon* species and cultivars employed in this study. A detailed description of the time-dependent processes involved in resistance is given for the host-parasite-system *Lycopersicon esculentum* cv. Hellfrucht and *Cuscuta reflexa*.

Within 10-12 days following infection the defence reaction became visible by brown spots on the stem surface of tomato just below the prehaustoria of the parasite, as already described by Ihl *et al.* (1988). No development of functioning haustoria was observed.

Microscopical analysis of the infection sites revealed a gradual enlargement (up to three times the normal size) of external host cells (belonging to the epidermis, hypodermis and collenchyma) below the parasitic prehaustoria followed by a compression of these cells and ultimate cell that (2-3 days following infection). Depending on the intensity of the defence, the cell layers below the collenchyma also reacted with a cell elongation (3-7th day), that pointed towards the infection site. The deteriorated walls of the elongated and subsequently collapsed cells formed the visible necrotic plaque surrounding the prehaustoria. These cell walls were not suberized or lignified, as evident from lack of reaction with Sudan III or phloroglucinol/HCL.

Cortical parenchyma cells showed tangential cell divisions (starting approximately at the 4th day of infection), which resulted in the formation of a hemispherical scalariform boundary tissue beneath the haustorial region. The involved cells were suberized and lignified, demonstrated by the positive reaction with Sudan III and phloroglucinol/HCl, respectively. Light-blue autofluorescence (excitation with 366nm-light) indicated incorporation of phenylpropanoids into these cell walls.

Introduction

Incompatibility of the obligate parasite *Cuscuta* with a variety of host plants was object of several works, especially in the last years. The diversity of findings suggest that different mechanisms may be involved in the defence reactions of incompatible hosts, for instance, expulsion of already differentiated haustoria from host tissue (Capdepon *et al.*, 1985; Schlenzka, 1992) or avoidance of parasitic penetration into host tissue, as shown for tomato infected by *Cuscuta reflexa* (Ihl *et al.*, 1988). Whereas the anatomical aspects of these defence reactions has been investigated frequently, knowledge about the involvement of primary and secondary metabolism in resistance towards *Cuscuta* is still limited.

Since a stimulation of phenylpropanoid metabolism have been documented for the defence reactions of numerous plants towards fungal or bacterial pathogens (Beimen *et al.*, 1992a and b; Bernards and Ellis, 1989) as well as during healing processes of injured tissue (Tronchet, 1969), we were interested in a characterization of the pronounced resistance of tomato towards *Cuscuta* with emphasis on the anatomy and on the possible involvement of phenylpropanoid metabolism.

The defence reactions, that were observed in thirty different *Lycopersicon* species and cultivars, were studied in detail for the incompatible relationship of *Lycopersicon esculentum* cv. Hellfrucht and *Cuscuta reflexa*.

Material and Methods

Plant material

Lycopersicon species and cultivars, *Cuscuta campestris* Yunker, *C. reflexa* Roxb., *C. platyloba* Progel were cultivated under greenhouse conditions with 15h and 9h darkness at 16-22C.
For infection experiments *Cuscuta* shoots (10-15cm length) were cut of the stock culture and twisted around wooden sticks to induce formation of prehaustoria. The time of subsequent transfer of these "activated" shoots to tomato stems (6-week-old) was defined as the onset of the infection (time zero). Artificial wounding of tomato plants (6-week-old) was performed with toothpicks piercing the stems.

Anatomical investigations

Transverse sections of infected or wounded tomato stems were observed under the light microscope using white light or 366nm (Leitz Ortholux II). For identification of suberin or lignin the sections were stained with Sudan III or phloroglucinol/HCL, respectively.

Preparation of tissue samples for phytochemical and enzymatic analysis

Samples from infected or wounded tomato stems were taken at various time intervals and prepared as follows: After removal of the *Cuscuta* (or the toothpicks) the healthy tomato tissue around the infection or the wounding site was cut off leaving the treated tissue as a segment of $2x2x3mm^3$ (IIT - Infected Internode Treatment site, WIT - Wounded Internode treatment site). For controls, tissue opposing the infection or wounding site (IIN - Infected Internode Neighbouring site, WIN - Wounded Internode Neighbouring site) and from the following internode towards the apex of treated plants (NI - Neighbouring Internode) was used. 8-16-segments from 2 tomato plants were pooled for each sample.

Phenylpropanoid analysis

Soluble phenolic constituents were extracted from freshly harvested tomato segments using methanol at room temperature.The methanolic extract was partitioned against petroleum ether to remove lipophilic compounds. The samples were subsequently centrifuged (10.000rpm, 5min), the

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ASPECTS OF THE INCOMPATIBLE HOST-PARASITE-RELATIONSHIP OF TOMATO AND *Cuscuta*

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ABSTRACT

Thirty different *Lycopersicon* species and cultivars were tested for their susceptibility to infection with *Cuscuta* spp. The pronounced resistance of the genus *Lycopersicon* towards the phanerogamic parasite, that was observed in all plants employed in this study, was characterized more detailed for the incompatible relationship of *Lycopersicon esculentum* Mill. cv. Hellfrucht and *Cuscuta reflexa* Roxb.

Microscopical analysis of the infection site (prehaustorial region) of tomato stems revealed formation of necrotic tissue surrounding the prehaustoria as well as suberinization of cell walls of living cells adjacent to the necrotic plaque. Preceding this phenomenon, an intensive enlargement of the external cell layers of *Lycopersicon* after contact with the adhesive secretory epithelium of prehaustoria was observed.

HPLC analysis of extracts from infection sites on tomato stems showed clear changes in the phenylpropanoid pattern restricted to the immediate haustorial region. An enhanced accumulation of chlorogenic acid as well as of a further unknown hydroxycinnamic acid derivative was demonstrated, whereas the concentration of the flavonol glycoside rutin remained largely unaffected by *Cuscuta* attack.

Excepting the enlargement of tomato-cells belonging to the outer cell layers, that is highly specific for the resistance towards *Cuscuta*, responses of *L. esculentum* similar to those observed following infection with the parasite could also be obtained by artificial wounding of stems.

Additional key words: induced defence, phenolics, peroxidases.

Table 1

FIXATION, GROWTH AND EMERGENCE OF *STRIGA HERMONTHICA* ON HOST ROOTS (FRAMIDA OR ARVAL). TWO POPULATIONS OF *S. H.* SEEDS WERE TESTED) MOKWA (NIGERIA) AND GEZIRA (SOUDAN)

Weeks after sowing	FRAMIDA				ARVAL			
	Stage 1	Stage 2	Stage 3	E	Stage 1	Stage 2	Stage 3	E
2	17 (2)	0 (2)	0 (1)		31 (32)	2 (21)	0 (6)	
4	30 (10)	10 (5)	7 (4)		15 (35)	20 (32)	15 (50)	0 (2)
6		15 (ND)	10 (ND)			18 (ND)	20 (ND)	1
8		ND	ND	0,2		ND	ND	5
10		ND	ND	1		ND	ND	8

Table 2

RADIOACTIVITY IN THE HAUSTORIA OF *STRIGA HERMONTHICA* FIXED ON *SORGHUM* THREE WEEKS AFTER SOWING AND IN THE HOST PLANT (BQ/HAUSTORIUM OR BQ/HOST PLANT)

	Stage 1	Stage 2	Stage 3	Host plant
Arval	0,9 ± 0,6	3 ± 1	19 ± 11	$6,7\ 10^4$
CK*	0,5 ± 0,25	3 ± 1,2	15 ± 9	$5,2\ 10^4$
Framida	0,35 ± 0,15	0,6 ± 0,25	4,6 ± 2,4	$6,1\ 10^4$

* In parasitizied Framida almost 50% of the haustoria haven't detectable radioactivity. They are not taken into account in the displayed results.
Five haustoria per sample were used for stage 1 and 2 with 6 repetitions.
One haustorium per sample was used for stage 3 with 15 repetitions.

Histological studies: Haustoria formed on Framida and susceptible varieties were studied 2, 3 and 4 weeks after sowing. There is little or no difference at 2 weeks. However large difference can be observed at 3 weeks (Fig. 3). In the case of susceptible varieties, haustoria have grown more rapidly and are larger. They are linked to host roots by tracheids that are more developed than in Framida and these tracheids are surrounded by a parenchyma whose cells are mainly filled with proteins identified by Mazia reagent (data not shown).

Uptake of ^{14}C-labelled compounds by the haustoria: A comparison of ^{14}C-labelled compound accumulation in haustoria shows that there is much less uptake from Framida roots than from CK and Arval (Table 2). Even at stage 3 (young subterranean stem with scale leaves), ^{14}C uptake is 4 fold higher in susceptible varieties than in Framida. Moreover, in parasitized Framida one haustorium out of two have not detectable radioactivity. There are many more differences in the ability of the haustoria on susceptible and Framida varieties to withdraw organic compounds from their hosts, than between their histological organization.

Reduce uptake mechanisms could be one explanation for Framida resistance to *Striga* .

2-D electrophoresis analysis of proteins in host root: Each 2-D gel contains over one hundred spots shown with a high resolution. Two weeks after sowing a 30 kD protein appears in the pattern of the parasitized FRAMIDA (Fig. 4 -A). This spot cannot be seen within the control (unparasitized FRAMIDA) (Fig. 4-B) nor with the parasitized and unparasitized susceptible varieties (ARVAL and CK-60 B). Two weeks later the spot disappears which leads us to the conclusion that it is a momentary accumulation of a specific gene product.

Concluding Remarks

Our results show that Framida resistance begins very soon after *Striga* seedling attachment. This allows us to discard some hypotheses about resistance mechanisms related to germination stimulant, root biomass or attachment. Resistant and susceptible varieties differ in their ability to prevent the forward development of a functional haustoria since metabolite flux from Framida roots to *Striga* haustoria were clearly demonstrated to be lower than those from CK or Arval. We found that one protein appears in parasitized Framida roots at the early stage of resistance. However, much more work will be required to demonstrate that this protein is a key product of a resistance gene. Other products of gene expression that could be overexpressed during the resistance stage are being searched by differential display reverse transcription polymerase chain reaction. These efforts could lead to evidence the gene(s) involved in Framida resistance.

Sorghum leaf n2 was abraded and soaked 10 hours in $NaH^{14}CO_3$ at 1 mM with 1.85 10^5Bq of total radioactivity, under a 150 mol. $m^{-2}.s^{-1}$ illumination, at 33 C. After a 15 h dark-period sorghum and subterranean *Striga* were separated. Radioactivity was measured in host plant and *Striga* haustorium.

Protein extraction and analysis: The roots to be analysed were separated from *Striga* seedlings, shoots, and haustoria. Proteins were extracted from roots of parasitized and non-parasitized (control) sorghum using the phenol-chloroforme method adapted from Camacho-Henriquez and Sanger (1982).

Electrophoretic analysis of proteins was performed following the procedure described by OFarrell(1975) with some modifications. Separation in the first dimension was in a 4% acrylamide gel containing 8M urea, 2% triton X100 and 5% (v/v) ampholines (4.5% pH 5-7, 0.5% pH 3-10) for 15 h at 400 V, followed by 2 h at 800 V. The proteins were then separated on a 12% (w/v) SDS polyacrylamide gel (Laemmli, 1970) for 4 h at 150 V. Proteins were vizualized by silver staining (BIO RAD) according to the manufacturers procedure.

Results and Discussion

Framida is a genuinely resistant variety as compared to CK-60 B and Arval since the number of *Striga* emerged 70 days after sowing is 8 times higher with susceptible varieties than with Framida (see Fig.3).

Striga seed germination: Percentage of germination of non-attached seeds of *S.hermonthica* was determined weekly after sorghum sowing. A slight decrease in this percentage was observed from the fourth week, but five weeks after sowing there were still newly germinated *Striga* seeds in pots. No significante difference among the three varieties were observed (Fig.1). The amount of germinated seeds results from both germination stimulant production and sorghum root development in pots. This led us to perform *in vitro* germination tests with root exudate, and to determine sorghum root biomass in pots.

In vitro germination tests show that Framida root exudate stimulated *Striga* germination in an intermediate range between CK-60 B and Arval (Fig.2). These results also demonstrate that the production of the *Striga* germination stimulant does not vary to a large extent during sorghum root development. On the other hand, no significant difference in the host plant growth could be evidenced between susceptible and resistant varieties, neither on shoot biomass nor on root biomass (Data not shown).

It could therefore be concluded that the resistance of Framida is not related to *Striga* germination, although other agroecological conditions might lead to results somewhat different from ours.

Striga fixation on host roots (Table 1). At the young haustorium stage (stage 1), the number of fixations on Framida roots are not inhibited and this clearly shows that the resistance of Framida does not occur at this stage. By contrast, at four weeks, haustoria developed as buds on host roots (stage 2) are more abundant in susceptible varieties than in Framida. A similar result can be observed with subterranean (stage 3) and emerged shoots (E). However it should be noted that such results are somewhat different when other populations of *S. hermonthica* seeds were used. Indeed, Framida roots were seen to be more resistant to *S. hermonthica* collected in Gezira (Soudan), even at the fixation on host root stage (Table 1). This could be regarded as an interesting case of virulence variation among *Striga* populations.

However in the first case (*Striga* seeds collected in Mokwa, Nigeria) we can conclude that the mechanism of Framida resistance to *Striga* takes place during the transformation of the young haustorium into a functional haustorium. Figures show that this change begins during the second and third weeks after sowing. This led us to investigate histological and molecular aspects of haustoria formation during this key period.

NTRODUCTION

rop resistance offers one of the best options for ontrol of *Striga*, since it is environmentally enign, requires no additional inputs and is otentially extremely durable. Sources of complete esistance to *Striga gesnerioides* (Willd.) Vatke ave been identified in cowpea (*Vigna nguiculata*), including varieties B301 and 58-57 Parker and Polniaszek, 1990). Cowpeas ncorporating resistance to *S. gesnerioides* have ecently been released to farmers in West Africa Atokpele *et al.*, 1995, B.B. Singh, pers. comm.). n *in vitro* system was developed to study the xpression of resistance of cowpea to *S. esnerioides* and two different mechanisms of esistance were characterised (Lane *et al.*, 1991; ane *et al.*, 1993). Both mechanisms were xpressed after penetration of cowpea roots by *S. esnerioides*; one resulted in the death of the arasite with associated host tissue necrosis round sites of parasite penetration, while in the ther, parasite infections failed to develop ormally. Related legume species, such as French ean (*Phaseolus vulgaris*), are resistant to *S. esnerioides* with an hypersensitive response eveloping around sites of parasite invasion (Lane t *al.*, 1994).

orghum (*Sorghum bicolor*) is the only cereal rop showing several examples of resistance to *triga*. The resistance of sorghum variety SRN 9 is caused by the inability of its roots to timulate parasite germination (Hess and Ejeta, 992). This variety is now released for control f *S. hermonthica* (Del.) Benth. in Sudan CRISAT, 1991). SAR sorghum varieties with his trait are also resistant to a red-flowered norphotype of *S. asiatica* (L.) Kuntze (Obilana t *al.*, 1991).

he aim of the present study was to assess hether phytoalexins (Vanetten *et al.*, 1994) were nvolved in the hypersensitive response of owpea and French bean roots infected by *S. esnerioides*. Phytoalexins are antimicrobial hytotoxic compounds which are synthesised by plants in response to infections and are thought to contribute to the resistance of plants to many microorganisms (Bailey and Mansfield, 1982). In addition, five SAR sorghum varieties were studied in order to determine the importance of post-infectional resistance mechanisms to *S. asiatica*.

Materials and Methods

Plant materials and phytoalexin extraction

Seeds of cowpea, variety 58-57, and French bean, variety Cannelini, were grown in vermiculite for seven days in a Fisons F600H growth cabinet at 30/25C (light/dark temperature), 67% RH with a 16 h daylength. Twenty plants of each species were transferred to the *in vitro* growth system of Lane *et al.* (1991). *Striga gesnerioides* seeds from Fada N'Gourma in Burkina Faso (LARS no. SG85-26) were germinated on host roots in the *in vitro* system and then 50 seedlings were placed on each root system. Copper sulphate ($5x10^{-4}$ M) was added to the roots of five uninoculated cowpea and French bean plants.

Host roots exhibiting hypersensitivity to *S. gesnerioides* infections seven days after inoculation were excised and separated into i) necrotic tissue, ii) host tissue from either side of the necrotic region and iii) parasite tissue which was external to the host roots. Copper-treated and untreated roots were also sampled at the same time. All samples were weighed and macerated in a small volume of 80 % ethanol for 10 minutes. Samples were dried, redissolved in acetone and applied to a silica gel TLC plate. Authentic samples of phaseollin, phaseollidin, phaseollinisoflavan, kievitone and 2'-methylphaseollinisoflavan were used as markers. The developing solvent was chloroform:ethanol (96:4). After development, plates were examined under UV at 274 nm and were sprayed with diazotized *p*-nitroaniline (DPNA) to detect phenolic compounds (Bailey and Mansfield, 1982).

V.4

POST-INFECTION RESISTANCE MECHANISMS AGAINST *Striga* IN COWPEA AND SORGHUM

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ABSTRACT

Resistance that is expressed during attempted infections of host roots offers great potential for the control of *Striga*. The existence of post-infection resistance mechanisms in the SAR sorghum varieties was studied, along with preliminary studies of the biochemical changes in the roots of infected legumes. The phytoalexins, phaseollidin, phaseollin and phaseollinisoflavan, were detected in the necrotic tissues of French bean (*Phaseolus vulgaris*) around the sites of infection of *Striga gesnerioides*. No phytoalexins were detected in the hypersensitive response of cowpea (*Vigna unguiculata*) variety 58-57 infected with *S. gesnerioides*, perhaps due to the low tissue weights available for analysis. A germination assay with five SAR sorghum varieties showed that there were large differences in their ability to stimulate *S. asiatica* (red-flowered colour morph) germination, ranging from 8 to 51% of a susceptible sorghum variety. The expression of host resistance after infection by *S. asiatica* was determined using an *in vitro* system. SAR 1 and 2 were completely susceptible to *S. asiatica*; thus, the resistance of these two varieties was entirely due to their reduced ability to stimulate parasite germination. In contrast, on SAR 13, 19 and 35 roots, parasite infections died or failed to develop normally. Resistance in sorghum is therefore based on several complex mechanisms, a characteristic which is very appropriate for the durable control of *Striga*.

Additional key words: durable control, genetic resistance.

Table 1

EFFECT OF THE SUNFLOWER PHYTOALEXIN COUMARINS SCOPOLETIN AND AYAPIN ON *O. CERNUA* SEED GERMINATION. DATA (MEAN OF THREE INDEPENDENT REPLICATES), PRESENTED AS PERCENTAGE OF GERMINATED SEEDS, WERE OBTAINED USING THE GERMINATION BIOASSAY ON PAPER

Chemical Stimulation	None	GR_{24}-INDUCED INHIBITION			INDUCTION	
		GR_{24} (lppm)	GR_{24} (lppm) + Scopoletin (1 mM)	GR_{24} (lppm) + Ayapin (1 mM)	Scopoletin (10 µm)	Ayapin (10 µm)
Germination (%)	0	86 ± 12	36 ± 8	43 ± 12	14 ± 7	16 ± 10

Table 2

O. CERNUA SEED GERMINATION ON AGAR IN THE PRESENCE OF ISOLATED SUNFLOWER ROOTS. THE EUROSEMILLAS (R), AND AGROSUR (S) VARIETIES AND THE BROOMRAPE POPULATION FLORASOL 92 WERE UTILIZED. DATA (MEAN OF THREE INDEPENDENT REPLICATES) ARE PRESENTED AS PERCENTAGE OF GERMINATED SEEDS

Variety	% GERMINATION				
Days	4	6	8	10	12
R	2 ± 1	18 ± 5	22 ± 8	23 ± 6	23 ± 8
S	5 ± 3	32 ± 8	38 ± 7	40 ± 8	43 ± 11

Table 3

LIGNIN AND COUMARIN CONTENT IN SUNFLOWER ROOTS INFECTED WITH O. CERNUA. THE AT0046 (4) VARIETY WAS USED FOR LIGNIN ANALYSIS AND THE EUROSEMILLAS (R) FOR COUMARIN CONTENT. SUNFLOWER PLANTS WERE INOCULATED WITH THE BROOMRAPE POPULATION FLORASOL 92. DATA OF A TYPICAL INDIVIDUAL EXPERIMENT ARE PRESENTE

DAYS Variety infection	LIGNIN (u.a.)		SCOPOLETIN ($\mu g\ g^{-1}$ tissue)		AYAPIN ($\mu g\ g^{-1}$ tissue)	
	N.I.	I.	N.I.	I.	N.I.	I.
7	0.75	0.82	0.90	2.78	2.62	6.75
14	0.93	1.18	0.20	0.48	9.00	10.40
21	0.91	1.42	0.30	1.80	4.15	5.80

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tissue, using the installation bioassay on plastic trays (Table III). Both, coumarins and lignins increased in response to the infection. In addition a higher excretion of coumarins has been detected (data not shown, Jorrín *et al.*, 1995b).

Conclusions

The bioassays described can be used to study sunflower parasitism by *Orobanche cernua* Loefl at the different stages of the life cycle of the parasite at biochemical and molecular level. Specifically we are using these bioassays to examine seed germination, haustorium formation, penetration and plant development, under controlled conditions. The culture methods allow discrimination for resistance/susceptibility between sunflower varieties and can be utilized for resistance screening, testing for chemical and biological control strategies of the weed and identification of host molecules that govern the life cycle of the parasite. Resistance to *Orobanche cernua* Loefl. seems to be associated to the induction in the host plant of defence reactions that avoid succesful establishement of the parasite more effectively than low or no production of germination and haustorium induction signals. Lignification processes and induction of the coumarins scopoletin and ayapin can be part of such defence reactions (Jorrín *et al.*, 1995b), although, in the susceptible host, xylem differentiation is also neccessary for parasite development. This hypothesis supports the idea that plants respond in a general way to different stress conditions, both biotic and abiotic, and opens new possibilities for the control of the parasite that implicate chemical or biological induction of the plant´s natural defences or their manipulation through genetic engineering techniques in order to obtain new crop varieties more resistant to the parasitic weed. In any case more direct evidence using plant mutants or by blocking the phytoalexin metabolism pathway using inhibitors of specific enzymes or silencing their genes is neccesary in order to probe their implication for the development of resistance to parasitic weeds.

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employed. Sunflower root fragments were used instead of germinating seeds. *Orobanche* germination rates, the number of aborted seeds, and germination distance from the root were quantified at different times of incubation. Both conditioned and non-conditioned broomrape seeds can be used with this bioassay.

Installation bioassay on plastic tray. The procedure is a modification of that previously reported by Lane *et al.* (1991) and is performed as indicated in de Ruck *et al.* (1995). The infection process was followed visually with a binocular microscope. At different time after inoculation, sunflower root tissue was intensively washed with tap water, broomrape seeds eliminated, dried with filter paper, frozen in liquid nitrogen and stored at -70 ºC for subsequent biochemical analysis.

Plate-installation bioassay. Sunflower plants were grown with sand in boxes with two plexiglas covers in a similar way to the described by Dörr *et al.*, (1994). When plants grew for 10-14 days conditioned or non-conditioned broomrape seeds were distributed homogeneously through the sunflower root. Plants were watered with nutrient solution (Tena *et al.*, 1984). The course of the infection was followed visually. The number of installed seeds and nodules and size of them were recorded.

Biochemical analysis. Coumarins and lignin content were determined in sunflower (susceptible and resistant) root tissue inoculated with germinated broomrape seeds (installation bioassay on plastic tray). The methodology for coumarin and lignin extraction and analysis has been reported by Gutiérrez-Mellado *et al.* (1995a,b) and de Ruck *et al.*, (1995).

Results

Germination bioassay on paper

Depending on the concentration utilized scopoletin and ayapin induced broomrape seed germination or inhibited GR_{24}-induced germination (Table I). When high phytoalexin concentrations (above 10 M) were added to GR_{24}, lower percentage germination was obtained, with both compounds acting sinergically. At low concentrations (below 10 M) they only slightly induced parasitic seed germination in the absence of GR_{24}.

Germination bioassay on agar

Orobanche cernua seeds were germinated in petri dishes containing agar in the presence of isolated sunflower roots. Germination was observed both with conditioned and non-conditioned seeds and in the presence of phenotypic-susceptible and -resistant sunflower varieties. A lower percentage of germination were obtained with the resistant variety (Table II).

Installation bioassay on plastic tray

This bioassay is very useful for following the infection process and tubercle development. Haustorium formation, adherence to the root and tubercle development (in number of 17 to 23 per plant, 25 days after inoculation) was only observed in the susceptible variety, but not in the resistant one. In the former case, the broomrape tissue necrosed 2 to 4 days after the haustorium adhered to the root.

Plate installation bioassay

10 to 30 days after the infection of the plants small tubercles appeared, in the order of 2 to 14 per plant in the susceptible but not in the resistant variety. Some tubercles, 1-3 per plant, grew to give mature *Orobanche* plants.

Biochemical analysis

The coumarins scopoletin and ayapin and lignins were analyzed in infected and non-infected root

Introduction

Despite its agronomical importance, and in contrast to *Striga*, little is known about the molecular processes that govern the life cycle of *Orobanche* and the molecular basis of host plant resistance or tolerance (Lynn and Chang, 1990; Parker and Riches, 1993; García-Torres *et al.*, 1994). This knowledge is strictly neccessary in order to develop control strategies for the parasite (Parker and Riches, 1993; García-Torres, 1994). To study the biological cycle of the parasitic weeds at the molecular level it is essential to employ bioassays in which the course of the interaction with their host plants can be followed under controlled environmental conditions. These bioassays must focus on the different stages of the host-parasite relationship (seed conditioning, germination, penetration and development) in order to distinguish between resistance and susceptibility at these stages. In addition they can be used as a quick test for chemical and biological control strategies. Different bioassays have been already published for plant parasitism, being utilized for both *Striga* and *Orobanche* (Lane *et al.*, 1991; Hess *et al.*, 1992; Muller *et al.*, 1993; Dörr et al., 1994; Van Hezewijk *et al.*, 1994). We have modified them to study of the sunflower interaction with *O. cernua* Loefl.

Although resistance to parasitic weeds has proven to be associated with host-cell necrosis (hypersensitive reaction, well studied and characterised in relation to plant-pathogen interaction) and different authors have suggested that phytoalexins (the coumarins scopoletin and ayapin, and the pterocarpans medicarpin and maackiain) biosynthesis and lignification processes can be an important part of the active defence mechanisms against parasitic weeds, a clear connection between the two facts has not firmly stablished and clear results in this respect have not been reported (Wegmann *et al.* 1991; Zaitoun et al., 1991; Antonova, 1994; Dörr et al., 1994; Lane et al. 1994; Wegmann, 1994; Zaitoun and ter Borg, 1994; Joel, 1995; Jorrín *et al.*, 1995a,b; Timko and Riopel, 1995). These defence reactions have proven to be effective against fungi and have been successfully manipulated for increasing resistance to pathogens (Lamb *et al.* 1992; Kessmann *et al.* 1994). Here we present preliminary results on the sunflower coumarins (Cabello *et al.* 1995; Gutiérrez-Mellado *et al.*, 1995a,b; Worsham and Klingman, 1962) and lignins (de Ruck *et al.*, 1995) indicating that the above mentioned hypothesis is correct and supporting the idea that plants respond in a general and indiscriminate way to different biotic and abiotic stresses, something that can be termed as biological cross-resistance. A more complete study will be published (Jorrín *et al.*, 1995b).

Material and Methods

Plant material

The sunflower varieties Agrosur (white seed coat, susceptible), AT0046 and Eurosemillas (black seed coat, resistant) were utilized. Seeds were germinated and grown as described previously (Tena *et al.*, 1984). *Orobanche cernua* Loefl. seeds were collected from sunflower infected fields (cv. Florasol 92). The susceptible or resistant character of the sunflower varieties against broomrape has been confirmed in our laboratory by using the installation bioassay on plastic tray and the plate-installation bioassay.

Bioassays

Four different bioassays were utilized.

Germination bioassay on paper. The technique described by Van Hezewijk *et al.* (1994) was followed with minor modifications. Scopoletin and ayapin were added alone or with GR_{24} to the petri dish after the conditioning period. Germination was determined using a binocular microscope. Data are expressed as percentage of germinated seeds.

Germination bioassay on agar. A modification of the procedure published by Hess *et al.* (1992) was

V.3

BIOCHEMICAL ASPECTS OF THE PARASITISM OF SUNFLOWER BY *Orobanche*

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ABSTRACT

Four different bioassays to study broomrape parasitism of sunflower under controlled environmental conditions have been optimized. These bioassays can be used: i) to investigate, at the molecular level, several key processes of the host-parasite relationship (seed conditioning, germination and penetration), ii) to test for broomrape resistance among commercial varieties of sunflower and other wild *Helianthus* spp., and iii) to discriminate for resistance at the different stages of the biological cycle of the parasite. All the sunflower varieties tested induced broomrape seeds germination, as revealed by a germination bioassay on agar with isolated roots. In contrast, differences between sunflower lines in supporting broomrape penetration and development has been observed using a plate-installation bioassay. The coumarins scopoletin and ayapin can, depending of their concentration, induce or inhibit broomrape seed germination, as shown by a germination bioassay on paper. Both scopoletin and ayapin as well as lignin content increased in sunflower roots in response to the inoculation with broomrape seeds, as indicated by a installation bioassay on plastic trays. Data presented here suggest that the phytoalexin coumarins and lignins can be involved in the resistance of sunflower against the parasitic weed *Orobanche cernua* Loefl. in a similar way to the resistance against fungi and insects.

Additional key words: plant defence reactions, biological cross-resistance.

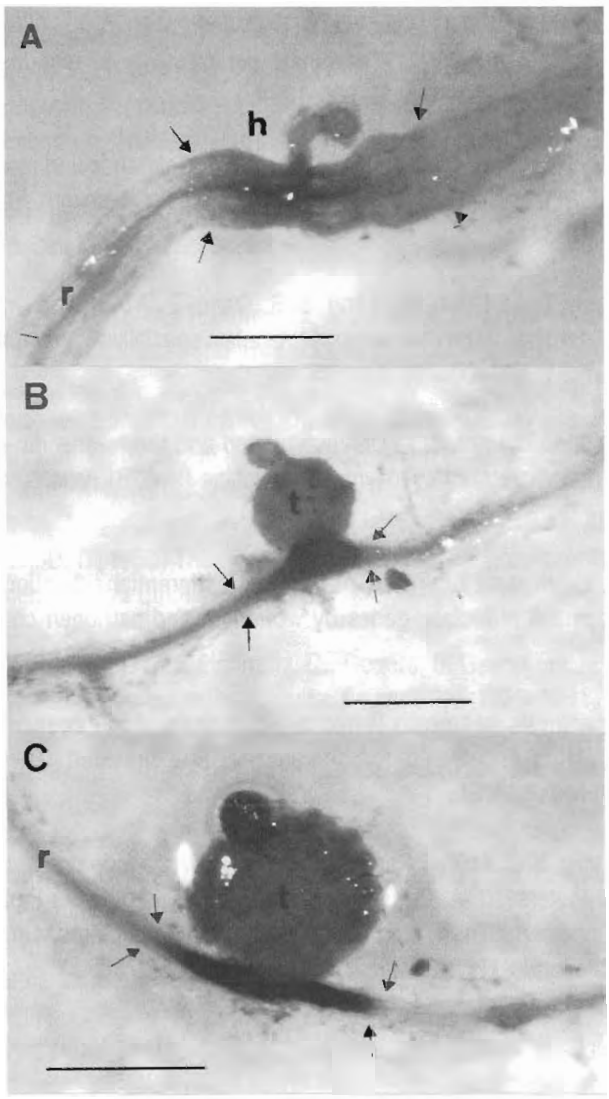

Figure 1

Transgenic tobacco plants containing *hmg2*:GUS gene constructs are successfully parasitized by *O. ramosa* (A) and *O. aegyptiaca* (B, C). The blue color indicative of GUS expression appears dark on the host root and is delimited by arrows. Bars represent 1 mm. h, haustorium; t, tubercle; r, host root.

Introduction

Despite considerable effort to identify and breed resistance to *Orobanche* into host plants, little is known about the hosts defense response to parasitism in either compatible or incompatible interactions. Several authors have characterized incompatible responses between *Orobanche* and hosts that show some resistance to attack and indicate a level of defense response on the part of the host, including the production of phytoalexins (Wegmann, *et al.*, 1991), and production of a physical barrier in the zone of penetration leading to tissue necrosis around the site of haustorial penetration (Dörr *et al.*, 1994; Goldwasser *et al.*, 1996; Zaitoun and ter Borg, 1994). Until recently it was not clear whether a susceptible host recognized the *Orobanche* haustoria penetration as a pathogen invasion, or whether the parasite was able to attach to the host root without alerting the host defense systems. Joel and Losner-Goshen (1994) reported that *Orobanche* parasitization of transgenic tobacco plants induced expression of a pathogen-response related gene promoter construct, PR-1b. PR1 is known to be induced by pathogenic microorganisms and elicitors as part of the systemic acquired resistance (SAR) response (Bol *et al.*, 1990; Eyal and Fluhr, 1991; Van de Rhee *et al.*, 1990; Ward *et al*, 1991).

3-Hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) is the first rate-limiting enzyme of the pathway leading to isoprenoid synthesis. It catalyzes the conversion of HMG-CoA to mevalonic acid (Bach, 1987) which is the precursor to numerous isoprenoid-derived compounds such as gibberellins, abscisic acid, carotenoids, plastoquinones, phytosterols, and phytoalexins (Bach, 1995; McGarvey and Croteau, 1995). The step catalyzed by HMGR is a primary regulatory point in the pathway and its activity is mediated by expression of differentially regulated HMGR genes in plants (Choi *et al.*, 1992; Yang *et al.*, 1991). In tomato, four genes encode HMGR and one isogene, *hmg2*, has been well characterized as the defense-specific gene (Weissenborn *et al.* 1995). The sesquiterpenoid phytoalexin products of the pathway are important in pathogen resistance and *hmg2* is induced by wounding, attack by pathogens such as *Erwinia carotovora* spp. *carotovora*, the nematode *Meloidogyne incognita*, and by elicitors such as arachidonic acid (Cramer *et al.*, 1993; Weissenborn *et al.*, in prep; Yang *et al.*, 1991). Analysis of pathogen induction of the tomato *hmg2* gene have been greatly fascilitated by use of transgenic plants containing a gene construct which fuses the *hmg2* promoter to a -glucuronidase (GUS) reporter gene. This experimantal system permits rapid histochemical analysis of both the temporal and spatial expression of genes in response to pathogens (Cramer *et al.*, 1993; Jefferson, 1987).

The availability of an *Orobanche*-susceptible host, which has been engineered to express a well-characterized pathogen response promoter, allows us to initiate studies on the molecular-level interactions between a parasitic plant and its host.

Materials and Methods

Transgenic plants of tobacco (cultivars NC 95 and Xanthi) were previously generated by *Agrobacterium*-mediated transformation (Horsch *et al.*, 1985) with a gene construct fusing 2.3 Kb of the tomato *hmg2* promoter with the GUS reporter gene (Cramer *et al.*, 1993; Jefferson 1987; Weissenborn, *et al.*, in prep, Yu, 1995). A Xanthi line was also transformed with the cauliflower mosaic virus 35S promoter (Koziel *et al.*, 1984) fused with the GUS gene and was used as a constitutively expressing control.

O. aegyptiaca and *O. ramosa* seeds were surface cleaned by washing in 70% ethanol for 30 seconds, 1% sodium hypochlorite solution for 10 minutes, followed by a triple rinse in distilled water for 10 minutes each time. Seeds were applied to glass fiber filter paper in either petri plates or polyethylene bags. Transgenic tobacco seedlings were surface sterilized and germinated on M.S. medium (Murashige and Skoog, 1962). Seedlings

V.1

THE HAUSTORIUM AND ITS DEVELOPMENT IN COMPATIBLE AND RESISTANT HOSTS

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ABSTRACT

This paper discusses the mechanisms of haustorium penetration, and refers to the possible host defence reactions that jeopardize parasitism. Changes in host cell organization correlates with both mechanical and enzymatic penetration. Evidence of pectinase involvement in penetration of *Orobanche* includes proofs that the pectin methylesterase is produced by the parasite in culture, that the enzyme is produced *in situ* during penetration, and also proof of depletion and chemical changes of the substrate of pectinase activity *in situ*. The latter two were obtained by an immunocytochemical study of the infection zone, using specific antibodies of pectin and pectic enzymes. After establishing a conductive connection with the host, the host adjusts to the presence of the parasite. In incompatible hosts the parasite seems to secrete an anchoring substance also in inner host tissues. Passive host defences include anatomical barriers and allelopathic prevention of parasitism. Stimulated resistance mechanisms develop during the attachment event and during haustorium penetration. A high competitive rate of host organs seems to determine host tolerance. Grafting experiments distinguish between resistance and tolerance.

Additional key words: Broomrape, *Orobanche* spp., parasitic weeds, incompatibility.

V.

Hosts Against Parasites: Resistance

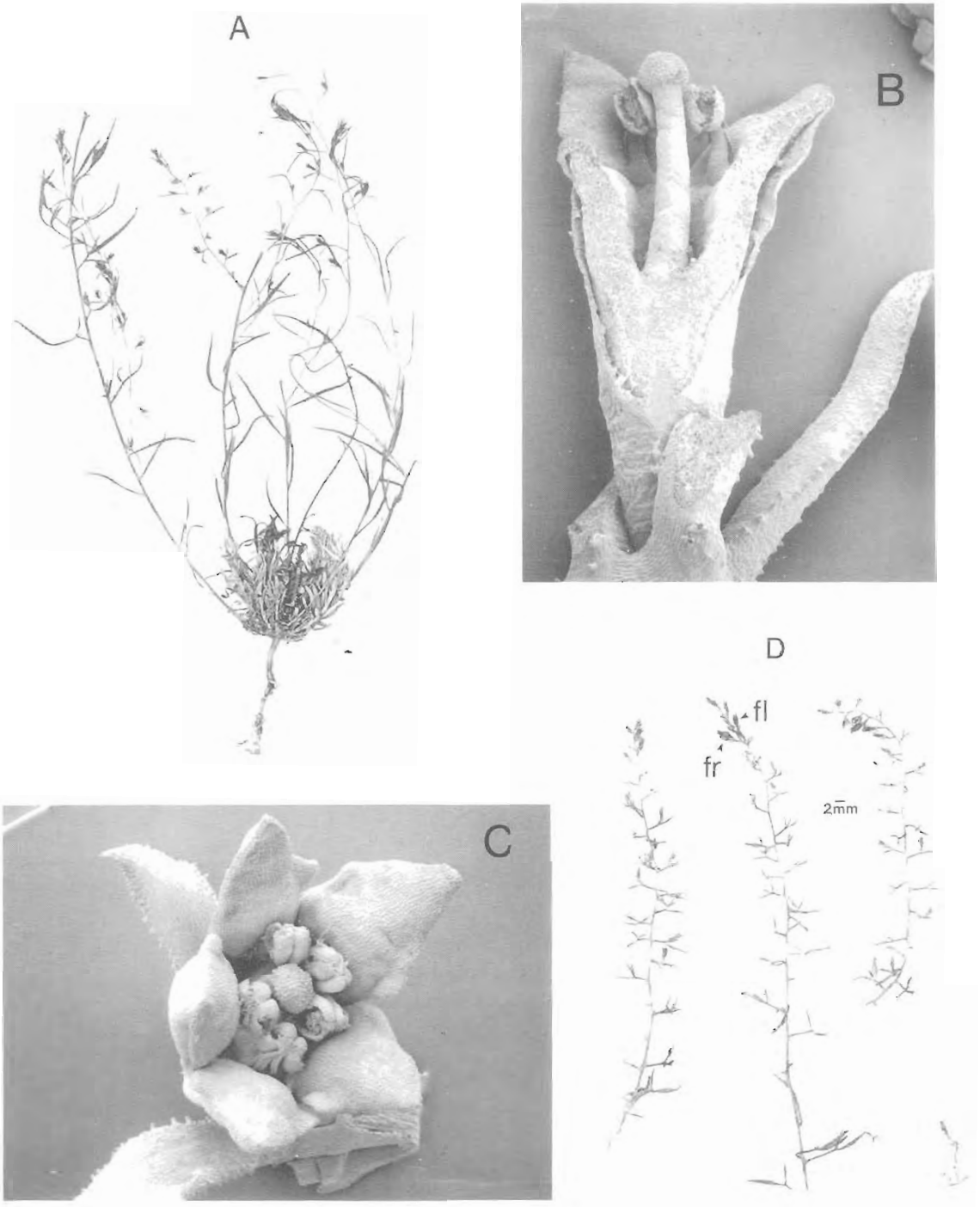

Figure 1

A-*Thesium aff. arvense* from Eddy County, North Dakota. E. S. DeKeyser, 529, ODU. B-C *Thesium* from Madison Country, Montana. L. J. Musselman, sn. ODU. Scale equal 1 mm. B-Longisection of flower. C-Flower showing the 5 parted perianth; bract at lower left, bracteole at upper left. D-Flowering and fruiting branch. Specimen from Eddy County, North Dakota. C. Slaughter, sn. ODU.

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IV.17

SANTALACEAE WITH WEED POTENTIAL IN THE UNITED STATES

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ABSTRACT

Pyrularia pubera, a native rhizomatous shrub, has been implicated in damage in a Christmas tree plantation in West Virginia. Symptoms included loss of leaves and branches and, in heavy infestation, death to the two or three year old trees. *Thesium*, an Old World genus and the largest in the family, was first collected in North Dakota in the 1940's and recently in Montana. Populations in both states are small and the same species. This has tentatively been determined as *T. arvense* although it is also similar to the Asian species *T. longifolium*.

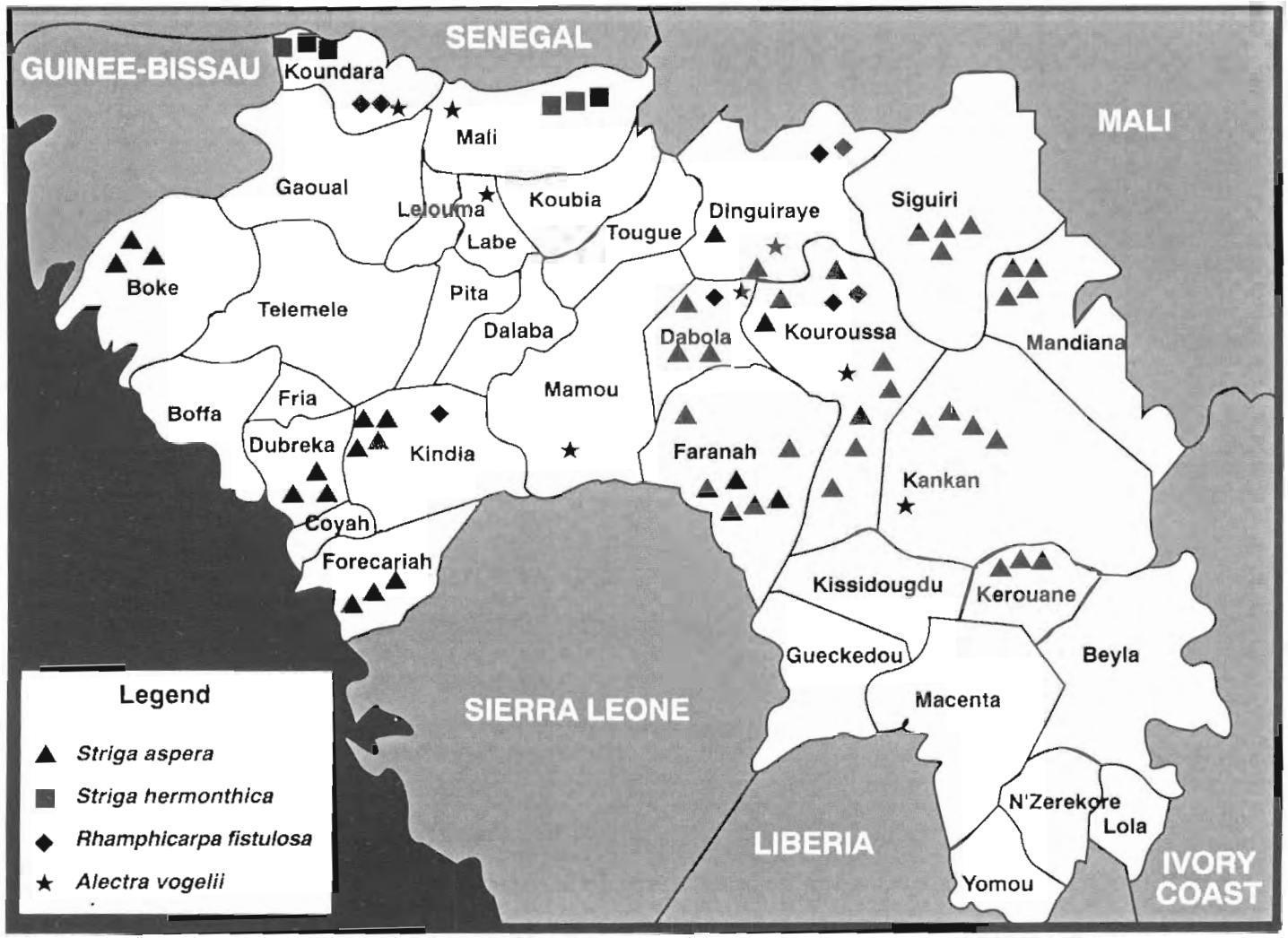

Figure 1

Distribution of *Rhamphicarpa fistulosa* and related species in Guinee.

IV.16

Rhamphicarpa fistulosa (SCROPHULARIACEAE) DAMAGES RICE IN GUINEE

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ABSTRACT

Rhamphicarpa fistulosa (Hochst.) Benth. is found in five Prefectures in Guinee. In some areas it causes severe damage to lowland rice.

Table 1

SHORT CARACTERIZATION OF THE INDICES OF ABUNDANCE FOR THE ASSESSMENT OF THE FREQUENCY OF *VISCUM ALBUM* SSP. *ALBUM* ALONG 8-10 KM OF ROAD PASSED THROUGH BY CAR. FOR CLOSER CHARACTERIZATION OF THE ABUNDANCE INDICES SEE METHODS

Index of abundance	Frequency of mistletoe
0	absent
1	very sporadic
2	rare
3	widespread, common
4	abundant
5	very large quantities

Table 2

DISTRIBUTION D (%) OF THE MISTLETOE ABUNDANCE INDICES

Index	D
0	20%
1	14%
2	29%
3	23%
4	12%
5	2%

may also depend on the direction taken to pass through a certain region. If one crosses for example a river associated with masses of mistletoe the index will be lower than if a road parallel to the river is pursued.

The presented distribution pattern of mistletoe does not cover the whole territory of France in a satisfactory density of registration points. Some peripheral parts in the north and north-west are not included at all. But the present map does show important geographic characteristics of mistletoe distribution and abundance in France.

Further studies to show correlations of mistletoe distribution with geological, climatic, hydrological and also biotic (birds, host trees etc.) factors are in progress.

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trees with many mistletoes or groups of trees with medium quantities of mistletoes at a few locations is classified as 3. Single trees covered with mistletoes at more or less regular distances or a few groups of trees with many bushes at a few places within the 8-10 km search was assessed as 4. The maximum index of 5 was introduced for large groups of trees covered with mistletoe occurring at shorter distances. We wish to stress the fact that the index numbers 0 to 5 do not correspond to a linear increase of mistletoe frequency. An illustration of the gradation of mistletoe abundance corresponding to the six described indices is given in Fig. 1.

Digitalization of data

As already mentioned, the indices of mistletoe abundance were directly noted into a road map. In order to allow a further evaluation of the enormous amount of data, the indices were digitized. This happened with the aid of a graphics tablet and special computer software ("Tracking", by A. Fritschy Umweltinformatik, CH-8031 ZÅrich, Switzerland).

Results

About 10.000 registrations of the abundance of V. a. ssp. album along the roads have been made in France. A distance of about 100.000 km was covered for this study.

The distribution of the different abundance indices is meaningful structured (Tab. 2). In 20 % of all observations no mistletoe could be detected. Index 2 was the most frequent. Very large quantities of mistletoe were rather seldom. This indicates that the classification of mistletoe abundance into six steps as used in the present study is accurately defined.

It is evident that *V. album* ssp. *album* is not uniformly distributed over the territory of France (Fig. 2). There is a decrease in frequency of mistletoe from the center of France towards the southern parts. North of a line La Roche/Yon – Poitiers – Montluáon – Villefranche/Saine – Geneva mistletoe can be found almost everywhere except for a few interspersed areas. Mistletoe is virtually absent in most coastal regions. The Beauce (between Paris and OrlÇans) represents one of the most conspicuous regions without mistletoe within this northern area .

In the southern parts of France, however, a few centers of diffusion of mistletoe lie within large areas lacking any mistletoe. Such clearly limited concentration centers of mistletoe could be detected in the Limousin, in the region of Saintes and of Bordeaux, and a smaller one could be found near Toulouse. Further south towards the PyrenÇes mistletoe is more frequent again.

The Bordeaux region has the biggest mistletoe concentration in the southern parts of France. But also in the central and nothern parts similar areas of mistletoe concentrations could be detected. The most important mistletoe centres lie in the Bresse, in the region of Langres, in a tract between St. Dizier, Troyes and St. Florentin, around Digoin (Charollais, Bourbonnais), around ChÉtillon/Indre and in the region of the Loire around Chinon.

It is evident that mistletoe accumulates along streams and rivers. Loire, Loir, Vienne. Indre, Cher, Armanáon, Seine, Aube are responsible for the linear course of some mistletoe occurrences, which traverse the landscape like veins (results not shown).

Discussion

The method for the evaluation of the frequency of *V. album* ssp. *album* presented here allowed the definition of diffusion centres of mistletoe and mistletoe free areas in France.

It should be noted that one index number represents the frequency of mistletoe on a linear distance. This means that the individual indices

Englerina lecardii (Mandinka: Sala-numbo) is a widespread species, more common in the western part of the country where it may be locally frequent. It is parasitic on *Combretum* species and is conspicuous in Sept./Oct. when the bright orange flowers are produced.

Tapinanthus dodoneifolius also parasitises *Combretum* species. Previously known from Senegal, Guinea, Guinea Bissau and Burkino Faso. It was recorded, as a species new to The Gambia, from the Kuntaur-Georgetown area, where it was locally frequent. This species produces its pink/cream flowers in Sept./Oct.

Berhautia senegalensis is an endemic species to West Africa (Balle, 1956), with a centre of distribution in the Tambacounda/Niokolo-Koba area of Senegal. It parasitises *Combrteum glutinosum* Per. ex DC. and was recorded in The Gambia at one site near Kuntaur, where five trees were infected. This discovery is the first localised record of the species in The Gambia and extends its range south into the country. It is possible that a more intensive search around the Kuntaur area would provide further records, the host being frequent in the area. *Berhautia senegalensis* is readily identified by its thick, leathery, roundish leaves and mauve flowers produced in Sept./Oct. Furthermore, it is the only West African member of the family with branched hairs on the stem and leaves.

Buchnera hispida was recorded as a weed in sorghum and maize in the Western Division although, at present, it is not considered to be of significant economic importance.

Striga hermonthica is the most widespread and common witchweed species and parasitises maize, millet, sorghum, upland rice and a range of indigenous grasses. Carson (1988a) has shown that of 700 fields surveyed, 75% were infested at a rate of 1-2 *Striga* shoots per m^2, sorghum being the most heavily attacked. Severe *Striga* infestation can result in total crop loss and therefore the weed is of major agricultural importance, with the only practicable control available to most Gambian farmers being hand pulling. *Striga aspera* is mainly parasitic on *Digitaria exilis* (Kippist) Stapf (hungry rice) a crop species which is grown in the east of the country. However, nowadays hungry rice is becoming less frequently grown because the grain requires too much preparation time before eating. *Striga gesnerioides* was only recorded in the Western Division in native vegetation. In other West African countries it is recorded as a serious agricultural weed on cowpea, a crop that is grown in The Gambia in the Upper River Division .

Conclusion

A total of 15 parasitic angiosperms have been recorded in The Gambia of which 9 were confirmed as a result of field work. Therefore, if it is accepted that The Gambia has between 1 000-1 600 angiosperm species, then between 0.9-1.5% are parasitic. According to Cronquist (1968) approximately 1% of the total world angiosperm flora is parasitic, thus, the figure for The Gambia fits into this overall picture.

In terms of number of species, *Striga* (Mandinka: Silo, Wollof: Ndohum) is the most well represented parasitic genus in The Gambia. This may be expected as *Striga* is predominantly an African genus with over half of the species being found in West Africa (Musselman, 1987). Gambians do not differentiate between witchweed species and use the same local names to include all members of the genera *Striga* and *Buchnera*, both being recognised as unwelcome crop parasites. There is less awareness of other parasitic species, as indicated by the lack of local names.

Since this survey has recorded two mistletoes new to The Gambia and both are accessible and conspicuous species this suggests that further botanical exploration could be rewarding. It is likely that additional parasitic species, particularly those known to occur in Senegal, may also be found in The Gambia.

SCROPHULARIACEAE
Alectra sessiliflora (Vahl) O.Ktze. var. *senegalensis* (Benth.) Hepper
Buchnera hispida Buch.-Ham. ex D.Don
B. leptostachya Benth.
Rhamphicarpa fistulosa (Hochst.) Benth.
Striga macrantha (Benth.) Benth.
S. forbesii Benth.
S. bilabiata (Thunb.) Kuntze. ssp. *rowlandii* (Engl.) Hepper
S. aspera (Willd.) Benth.
S. hermonthica (Del.) Benth.
S. gesnerioides (Willd.) Vatke

A record not cited by Hutchinson and Dalziel (1954-1972) was that of Jarvis (1980), who listed *Englerina lecardii* (Engl.) Balle (Loranthaceae), yet, data was not provided on the location of voucher material. In addition, Terry (1981), made reference to *Striga asiatica* (L.) O. Ktze. (Scrophulariaceae) occurring in The Gambia as a weed of upland crops, but he was unable to confirm any records.

Study of herbarium material provided an unlocalised record of *Berhautia senegalensis* Balle (Loranthaceae) from northern Gambia held at **K**. Whilst the herbarium housed at the Dept. of Livestock Services contained specimens of *Englerina lecardii* to support the information in Jarvis (1980).

Field work confirmed 8 of the above records and added *Tapinanthus dodoneifolius* (DC.) Danser (Loranthaceae) to the species list, however, I was unable to confirm records for *Alectra sessiliflora*, *Buchnera leptostachya*, *Rhamphicarpa fistulosa*, *Striga macrantha*, *S. forbesii*, *S. bilabiata* and *S. asiatica*.

Discussion

The following species were recorded during field work:

Cassytha filiformis is a pantropical species usually growing amongst coastal vegetation. In The Gambia it is locally frequent along the coast, particularly south of the river, although it is also found upriver as far as Georgetown. It has a wide host range of trees and shrubs and a heavy infestation may cloak the host. The parasite flowers in Dec./Jan., however, the open flowers look unopened.

Tapinanthus globiferus (West African mistletoe, Mandinka: Dungo, Wollof: Bentenkeh) is the most widespread mistletoe species in The Gambia. It occurs throughout the country, although is more frequent in the Western Division. It has a wide host range of both native and introduced trees and is particularly common on baobab (*Adansonia digitata* L.), *Albizia lebbeck* (L.) Benth. and *Citrus* species. Heavy infestations may kill the host and *T. globiferus* could become a serious weed species if *Citrus* was grown in The Gambia on a commercial scale. The mistletoe is a serious pest species in other West African countries, for example on cocoa in Ghana (Phillips 1991) and on shea trees in Burkina Faso (Boussim *et al.* 1991). Although the plants can be found in flower throughout the year, there is a noticeable flowering period in Jan./Feb. Field observations suggest that the flowers are primarily pollinated by sunbirds, the seeds are also bird dispersed.

According to Hallam (1978), mistletoe growing on *Pterocarpus erinaceus* Poir (Gambian rosewood or keno- *Fabaceae*) can be used in a rite to obtain protection from a person with evil intentions. "One first has to make a cross from dry grass stems. Then, climb the KENO tree naked late at night and light the three ends of the grass under the out-growing DUNGO. Collect the ashes of the burned parasite, take it home and when the first cock crows the following morning, put the ashes into some water and drink it". In addition, Hallam (1979) states that dried mistletoe leaves and flowers can be used to "get rid of worms" and to treat "swollen body diseases". He also reports that powdered leaves can be mixed with seeds when sowing and act as a fertiliser.

IV.14

PARASITIC FLOWERING PLANTS OF THE GAMBIA

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ABSTRACT

This paper is an overview of the species of parasitic angiosperms recorded in The Gambia. The methodologies employed were a literature review, herbarium searches and field work. The literature review established that 13 parasitic species have been recorded from the families: Lauraceae (1 species), Loranthaceae (2 species) and Scrophulariaceae (10 species including 6 *Striga* species), with each record supported by a voucher herbarium specimen. Field work confirmed the existence of 7 of the species cited in the literature. In addition, two mistletoes, *Berhautia senegalensis* and *Tapinanthus dodoneifolius* were recorded. The distribution of each species recorded during field work is provided, along with information on their ecology and ethnobotany.

Introduction

Broomrape, *Orobanche cernua* Loefl. is a serious root parasite on tobacco crop in Andhra Pradesh India. It debilitates the crop growth resulting is considerable loss in yield and quality. In spite of extnsive studies on the parasite, its control aspect present considerable difficulties (Wegmann and Musselman 1991). The parasite is also found to attack other crops such as eggplant, tomato and safflower in this region. But there is no information on the relative susceptibility of these crops under local conditions to enable to categorise their susceptibility for utilization in studies on management of the parasite. Hence, the relative susceptibility of these crops was studied under field conditions and the results are presented in this paper.

Materials and Methods

One broomrape sick field was selected and seeds of *O. cernua* Loefl. were inoculated at 100 mg/m^2 as additional inoculum of the parasite. The field was plughed and seedlings of *Nicotiana tabacum* (variety CTRI Special), *Solanum melongena* (eggplant - local) and *Lycopersicon esculentum* (tomato - local) were transplanted at 100 x 80 cm spacing in blocks, each block accomodating 1000 plants of each crop. In case of safflower (*Carthamus tinctorius* - local), the seeds were dibbled at 10 x 20 cm spacing. At two months age, observations were taken on the incidence and intensity of broomrape on all the plants of each crop. In addition, 50 plants of each crop were scooped out, the root system washed carefully in water and examined for the condition of subterranean infection on the roots. The data are presented in the table.

Results and Discussion

Tobacco, tomato and eggplant crops showed 100% incidence of the parasite with an intensity of 26, 28 and 15 emerged shoots per plant respectively (Table 1). On these crops, subterranean infection of different stages were also noticed; pin head sized (1-2 mm dia') infections being 42, 45 and 20 per plant, button sized (3-5 mm dia') infections 23, 21 and 7 per plant and finger shaped shoots (1-5 cm long tentacles) 10, 12 and 5 respectively. The condition of all these subterranean infections appeared healthy. In case of safflower crop, out of 4000 plants, only one broomrape shoot was noticed. The condition of this single shoot was weak and it died slowly without flowering. On this crop, 20 pin head sized infections and 2 button sized infections were observed on the roots and these died.

Results clearly indicated that tobacco, tomato and eggplant are highly susceptible, while safflower turned out to be a falsehost, with the presence of dead pin head, button sized infections and rarely emerged single unhealthy shoot which died subsequently without flowering. The results of this field study are in agreement with those of earlier pot studies from this Institute (Krishna Murty *et. al.*, 1977). Kropez (1973) mentioned that certain hosts induce the formation of subterranean clusters of tentacles without subsequent emergence of flowering shoots. It is hoped that safflower acting as false-host in this study would be of use in studies on the management of the parsite in broomrape sick fields.

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Table 2

HOSTS AND ASSOCIATED *OROBANCHE* SPECIES RECORDED IN DIFFERENT GEOGRAPHICAL LOCATIONS IN DELTA, MID AND UPPER EGYPT, 1994-1995

LOCATION (1)	HOSTS	*OROBANCHE* SPP.
A1	Broad bean, field pea, carrot	*O. crenata*
A2	Broad bean, field pea, dill	*O. crenata*
	Cauliflower, cabbage, egg plant, lettuce	*O. aegyptiaca*
	Tomato	*O. ramosa*
A3	Broad bean, clover, sow tistlle, polygonum, trigonella, sugar beet	*O. crenata*
	Cauliflower	*O. aegyptiaca*
A4	Broad Bean	*O. crenata*
A5	Peas	*O. crenata*
B	Chamomile	*O. crenata*
C	Broad bean, peas, artemisia, clover demsisa, celery, lathyrus nasturium lentils, caraway, amaranthus garden cumin, chick peas	*O. crenata*
	Clover safflower, lupinus, sow thistle	*O. minor*
	Broad bean, lupinus	*O. cernua*
	Tomato	*O. ramosa*

(1) See Materials and Methods.

Table 1

OROBANCHE PARASITISM UNDER COMPARATIVE AGRICULTURAL CONDITIONS IN INFESTED LOCATIONS IN DELTA AND UPPER EGYPT (1993-1994)

RECORDS	NORTH DELTA	MIDDLE DELTA	UPPER EGYPT
Hosts	Peas	Eggplant, Egyptian clover cabbage cauliflower, tomato potato	Faba bean Egyptian clover carrot tomato cumin chick pea lupin sweet pea chamomile garden nasturium
Soil type	Sandy new reclaimed	Heavy clay-old soil	Heavy clay old-new reclaimed
Sowing dates	October	July to Nov.	Feb. and Spt. to Nov.
Broomrape emergence	December	Oct. to March	Jan. to March.
Irrigation	Mist	Surface irrigation	Surface irrigation

IV.12

HOSTS OF *Orobanche* SPP. AND YIELD LOSSES IN DELTA AND UPPER EGYPT

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ABSTRACT

Orobanche spp. were recorded on leguminous crops, specially faba bean and peas, and on solanaceous and cruciferous vegetables in five governorates in Delta. In Upper Egypt *Orobanche* spp. were mainly recorded on leguminous and umbelliferous crop plants. Broomrape infection was also observed on clover and some dicotyledonous weeds. In the surveyed areas the infection ranged from the minimum of 18% in Upper Egypt to a maximum of 86% in North Delta. Yield losses differed according to the geographical site, infestation percentage, infested crop and farmers attention to control. Yield losses ranged between a minimum of 7% to a maximum of 80%. The observations indicated the broadening of host range to include economically unimportant new plant genera and species. It also showed spread of infection from the old soil in the Valley towards the new reclaimed areas, and from the Delta in north Egypt towards the south in Upper Egypt.

Table 3

STRIGA DENSITY IN 76 INFESTED FIELDS (MEANS OF OVERGROUND *STRIGA* SHOOTS PER M^2 OR PLANT STAND

Number of *Striga* shoots per m^2 plant stand	Proportion of infested fields	Proportion of rice fields	Proportion of maize fields
1-2	19%	21%	15%
3-5	29%	43%	29%
6-10	44%	36%	41%
> 10	8%	–	15%

Table 4

STRIGA DENSITY IN 76 INFESTED FIELDS (MEANS OF OVERGROUND *STRIGA* SHOOTS PER M^2 OR PLANT STAND

Control method	Proportion farmers (%)	Estimation positive effectiveness (%)	Estimation negative effectiveness (%)	No stament statement statement (%)
Weeding	30	42	47	11
Fallow	16	40	10	50
Fallow + Weeding	10	83	17	
Fallow + Manure	9	50	17	33
Weeding + Manure	9	100		
Weeding + Crop rotation	7	100		
Fallow + Weeding + Manure	3	100		
Falllow + Manure + Cotton	2	100		
Fallow + Cro protation	5	50	50	
Weeding + Intercropping	2	100		
Early sowing + Weeding	3	100		
No control	4			

Table 1

PERCENTAGE OF FIELDS INFESTED WITH *STRIGA ASIATICA* IN THE NORTHERN MIDDLE WEST OF MADAGASKAR

	RICE		MAIZE		RICE AND MAIZE INTERCROPPED		OTHERS*	
Percentage of infested plant stands	Absol.	Relat. %	Absol.	Relat. %	Absol.	Relat. %	Absol.	Relat. %
0	17	56	38	42	12	70	1	14
<10	5	17	10	11	0	0	2	29
10-49	5	17	25	28	3	18	3	43
50-74	1	3	9	10	2	12	1	14
75-100	2	7	8	9	0	0	0	0
Number of surveyed fields	30		90		17		7	
Proportion of infested fields	13	44	52	58	5	30	6	86
Mean percentage of infested plant stands		13		21		13		29

* *Rice or maize intercropped with legumes (groundnut or bambara groundnut).*

Table 2

STRIGA INFESTATION AND INFECTION IN DIFFERENT DISTRICTS OF THE MIDDLE WEST, MADAGASCAR 1993

District	Proportion infested fields (%)	Mean percentage of infested plant stands[1]	Percentage of infested plant stands	
			Minimum	Maximum
Analavory	41	39	2.5	95
Ankadinondry	23	38	2.5	60
Bemahatazana	75	44	2.5	100
Mahasolo	91	36	2.5	75
Miandriarivo	50	25	2.5	75
Tsinjoarivo	75	28	2.5	75
Tsiroanomandidy	54	45	2.5	75

1 *Infested plant stands were considered only.*

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Striga shoots, other weeds, diseases and insects can not be taken into consideration (Sallé *et al.*, 1995).

Generally, 78% of farmers stated an increase of *S. asiatica* infestation for several years because of an increasing number and size of infested spots in their fields. 58% of farmers already abandoned fields due to high *S. asiatica* infestation.

The parasitic nature of *Striga* was unknown to the farmers. Many farmers believed that *Striga* poisons the crop so that it cannot grow. The knowledge about *Striga* multiplication was low and the majority of farmers were not aware that *Striga* propagates by seeds. 64% of farmers could not give a reason for the spreading of *Striga*, 14% presumed *Striga* to grow on exhausted soils and 9% supposed the utilization of pesticides or dirty seed to be the reason for the spreading of *Striga*.

According to the farmers, weeding by hand is the most prevalent strategy to control *Striga*. However, they consider the effect very variable. Half of the farmers who practice weeding as a single control method (30%), could not observe a decrease of the *S. asiatica* infestation. They weed during or after the flowering of *S. asiatica*. A reason for the low effectivity of hand weeding could be the re-infestation with *Striga* because only 15% of farmers remove weeded *Striga* plants to the field borders where they burn or bury the *S. asiatica* shoots.

16% of farmers fallow their infested fields but little information was given about the impact on following *Striga* infestation level. According to calculations of Kunisch *et al.* (1991) first after 14 years of fallow 95% of the *Striga* seed bank in the soil is reduced. 10% of farmers realized that the period of fallow was too short in order to effectively control *Striga*. After 2 years of cultivation of cereals, the *S. asiatica* infestation reached its preceeding level. 11% of the respondents do not fallow their land due to a lack of sufficient fertile land. After all, fallowing is a double-edged control measure because the ground cannot be used.

Organic manure is used by 86% of farmers not as a targeted control method but to maintain the soil fertility and to increase the crop vigor. Farmersí opinion on the resulting impact of *Striga* infestation was varying. Where the applied amount of manure exceeded 5 t/ha, no further increase of *Striga* was observed. The average amount of applied organic manure amounts to 3.5 t/ha (corresponding 17.5 kg N/ha) and is not sufficient to suppress *Striga* infestation. On the contrary, lower N-levels (14 kg N/ha) were found to increase *Striga* emergence (Ramaiah and Parker, 1982). Several farmers observed an increase of *Striga* infestation because of seed propagation by cattle. The transport of the manure is a constraint when fields are located far away from the villages.

Combining of different control methods is used by 50% of farmers. 10% of these farmers fallow their ground and weed *Striga* (Table 4). 9% of farmers considered weeding and the use of well-rotted organic manure as a promising control method. They observed a decrease of *Striga* infestation after several years.

Experience by many workers in the past indicated that no single control method can effectively control *Striga*. Integrated *Striga* management practices have been recommended in countries of Africa (Ogborn, 1977). For future control strategies, farmers should be trained in possible control methods and *Striga* biology.

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Here, *S. asiatica* plants were smaller compared to those parasitizing on maize or rice. Host weeds were *Rottbellia exaltata* (L.), *Cynodon dactylon* (L.) Pers., *Digitaria biformis* Willd. and *Echinochloa colona* (L.) Link.

S. asiatica was represented on brown-red ferrallitic, acidic (pH 4.5-5.5) clay loam soils but sporadic presence could be found on alluvial clayey-sandy soils as well, which is an indication of its capacity to adapt to different ecological conditions. Similar observations could be made by Sullivan (1985) in Nigeria and Benin.

About 53% of the 144 inspected fields were infested by S. asiatica with an average infestation intensity of 17%. In maize, the infestation rate (58%) was higher compared to upland rice (44%). On average, 21% and 13% of the plant stands in maize and upland rice were infected, respectively (Table 1).

The highest proportion of infested fields was found in the district of Mahasolo with an infestation of 91% and 75% for Bemahatazana and Tsinjoarivo, respectively (Table 2). In the district of Bemahatazana the maximum infection intensity of 100% was found in single fields.

About 44% of fields showed an average rate of 6-10 *Striga* shoots per m2 or per plant stands (Table 3). 8% of fields were severely infested with more than 10 shoots per m2 or per plant stand. Maize fields were stronger attacked by *Striga* than rice fields.

In the farming systems of the interviewed farmers maize, lowland rice and upland rice were by far the most dominant crops. Maize and lowland rice each occupied 30%, while rainfed rice occupied 20% of the total field area. Cassava covers only 7% of the crop area, whereby bambara groundnuts (*Vigna subterranea*) and peanuts covered less than 7% of the total field area. Sweet potatoes, beans (*Phaseolus vulgaris*), tomatoes, pumpkins (*Cucurbita* spp.), cotton, sugar cane and tobacco are crops of minor importance. Vegetable gardening is restricted to more fertile regions at the border of the surveyed region. Maize and upland rice are mostly monocropped. However, maize is also intercropped with cassava, bambara groundnut or peanut. Farmers practice various crop rotations on upland fields. Maize or upland rice is often planted first after a fallow, followed by legumes or cassava as third- to fourth-year crop. Very often subsequent cultivation of maize and rice during several years occurs. Fields are fallowed after 3-5 crop years. Although the population density of 15 inhabitants per km2 is low (Anonymous, 1991), farmers reduced the fallow periods (Fujisaka and Fofifa, 1990). Fallow periods last between 1 and 5 years, with the average being 2 years.

88% of the farmers interviewed apply fertilizer in the form of cow manure in maize and upland rice fields, 12% apply mineral fertilizer. The application of pesticides is limited to seed treatment against soil born insects (59% of farmers). Inputs such as mineral fertilizer and herbicides are still little used because of economic reasons.

From the farmers' point of view *S. asiatica* is considered to be the most important pest problem besides *Heteronychus* spp., where the larvae and adults are damaging the roots and sprouts of maize and upland rice. Farmers in the surveyed area all recognized *S. asiatica* from photographs. In general, *S. asiatica* is called "arema", which is the malgache translation for red. 88% of the interviewed farmers know *S. asiatica* from their own fields and 44% considered *S. asiatica* as a main problem in grain production. They all estimated crop losses due to *S. asiatica* between 30 and 90%, depending on the sowing date. With early sowing of maize before the beginning of the rainy season, they often observe less crop losses compared to later sowing dates in December. The time of *S. asiatica* infection is an important factor influencing the degree of crop losses. Egley (1971) showed that early infection of *Striga* resulted in higher crop losses compared to late infection. However, exact calculations of crop losses remain difficult because the influence of subterranean

Introduction

The semi-parasitic weeds of the genus *Striga* are well known as a constraint to grain production in the savannah regions of Africa. Introduced from the African continent to the islands in the Indian Ocean, *Striga asiatica* (L.) Kuntze, parasitizing cereals such as maize, sorghum, millet and rice as well as sugar cane, has been spreading in Madagascar's Middle West for several years.

Rice and maize, produced in low- or upland rainfed ecologies, are the most important crops in Madagascar's Middle West, one of six major rice-growing areas (Fujisaka and FOFIFA, 1990). The Middle West covers 23.500 km2 lying at an elevation between 700 and 1000 m above sea level. The climate is semihumid tropical, with a warm, rainy season from November to April and a cool, dry season from June to October. The annual rainfall ranges from 1100 to 1900 mm and the mean temperature is 22°C. Upland areas ("tanety") planted to maize, rice, cassava and legumes cover approximately 60-70% of the arable land in the Middle West (Anonymous, 1991). For several years, farmers in the Middle West have complained about decreasing yields due to an increasing infestation of *S. asiatica* in upland rice and maize. Little detailed information is available on the distribution and degree of importance of *S. asiatica* in this region. To evaluate the magnitude of this problem, a field survey was carried out in January/February 1993 during the growing season. In addition, interviews were conducted to identify cropping and/or cultural practices and their possible impact on the distribution and the severity of *S. asiatica*.

Materials and Methods

The survey was carried out around villages in the districts of Analavory, Ankadinondry, Bemahatazana, Mahasolo, Miandriarivo, Tsinjoarivo and Tsiroanomandidy before the flowering of *S. asiatica*. Ninety fields of maize, 30 fields of upland rice and 24 fields of maize or rice intercropped with legumes were examined. Along the main roads, every 3-5 km fields with advanced crop development were randomly selected.

On 20 randomly selected plant stands of maize or 20 m2 of rice, respectively, the infection intensity (proportion of attacked plants per field) was determined using the sampling method described by Wetzel (1984). Beginning at the field border, the field was crossed in a zigzag course and *Striga* shoots were counted in regular distances around the plant stands. The values of *Striga* infection intensity were summarized in 5 infection classes. In rice, the sample area was one square metre, whereas on the maize fields the sample area was 1,1 m2 around the maize plant.

In addition, a questionnaire was conducted among 73 randomly selected farmers. The questionnaire consisted of questions concerning the farming system, general plant protection problems and in particular the *Striga* situation. The questionnaire was pretested and adjusted. The interviews were carried out by collaborators of the local extension service.

Results and Discussion

During the growing season 1992/93 the first *S. asiatica* plants on maize were observed in December. The maximum infestation level occurred in February and March. According to extension workers and farmers the emergence of *S. asiatica* was later as usual and was probably related to the irregular rainfall patterns at the beginning of the rainy season.

Only the red coloured *S. asiatica* was found in the Middle West. In highly infested fields, *S. asiatica* was well scattered compared to low infested fields, where plants were mainly found at the field borders. Heavily infested rice and maize showed yellowish-green leaves and stunted growth. It was often observed that heavily infested maize prematured. *S. asiatica* was also found on spontaneous vegetation, fallow and rangeland.

Table 1

STRIGA AND HOST MATERIALS USED IN THE EXPERIMENT

LOCATION	*STRIGA* HARVESTED ON		HOST CROP LANDRACES	
	Millet	Sorghum	Millet	Sorghum
Bengou	x	x	Hainikiré de Bengou	Bengou local
Guéza	x		Guéza millet	Guéza sorghum
Sadoré	x		Sadoré local	
Birni N'Konni	x	x	Guré-Guera	Mota Galmi
Maradi	x		Zongo local	Mota Maradi

Figure 1

Striga hermonthica collection sites in Niger (*Striga* harvest in 1991).

P.V.P. 5.700