CONGRESOS Y JORNADAS 51/99

RESISTANCE TO OROBANCHE: The state of the art

COMUNIDAD EUROPEA

Consejería de Agricultura y Pesca

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Publica: Dirección General de Investigación y Formación Agraria. Servicios de Publicaciones y Divulgación.
Colección: Congresos y Jornadas 51/99
Autores: José I. Cubero, Mª Teresa Moreno, Diego Rubiales, Josefina Sillero.
Fotografías e Ilustraciones: Autores
I.S.B.N.: 8489802-51-3
Depósito Legal: SE. 1883 - 99
Fotocomposición e Impresión: J. de Haro Artes Gráficas, S.L. Parque Ind. P.I.S.A. Mairena del Aljarafe • Sevilla.

RESISTANCE TO OROBANCHE:
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STUDIES ON RESISTANCE TO *Orobanche crenata* IN *Vicia faba*

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1. Genetics.

Tables 1 and 2 summarize respectively the papers on the genetics of *V. faba* to *O. crenata* published until now and the main data derived from segregating generations. The main conclusions obtained from these papers can be summarized as follows:

Additivity is always very strong, very frequently being the only genetic component; a similar conclusion was also obtained for the system *V. sativa/O. crenata*, although weak additive x additive interactions were detected (Gil *et al.*, 1987).

When dominance effects are present, susceptibility is usually dominant over resistance; dominance, if present, is always partial; however, there are cases where resistance is dominant over susceptibility. There is at least one case where there was genetic complementation: a cross between two very highly susceptible lines gave a certain proportion of resistance in F2 and F3.

This genetic heterogeneity for resistance has to be a consequence of the coexistance of different resistant mechanisms. Thus, genes for resistance can be found in many different lines and can be either dominants or recessives.

There are biotypes of the parasite showing differential agressivity. The parasite x host interaction is very small.

Resistance seems to be not race-specific (horizontal *sensu* Van der Planck). Thus, is possible to use resistant lines in a wide range of environments.

2. Selection index.

The number of broomrape shoots per host plant seems to be the most reliable one, as in many other crops. At the present time, each plant or line to be tested is surrounded by four plants (or clusters) of a common susceptible check; the number of bromrapes in the observed genotype is then referred to the average of the four plants/clusters of the susceptible check. In this way, environmental effects such as the possible infestation heterogeneity of the experimental field are reduced.

A better index would be the total number of *Orobanche* plants attached to the roots of the host, very difficult to be recorded under field conditions, but there is an extremely high correlation between this number and the total number of *Orobanche* shoots emerged after plant maturity (Cubero, 1983).

A logarithmic transformation is required for statistical analysis when individual counts (i.e., broomrapes per plant) are used as the distribution of this index follows a Gamma distribution (Cubero, 1983). Because of this distribution, plants to be selected must not only show zero broomrapes but must also belong to a family with a high proportion of zero-broomrape plants: most F2 or F3 families always give a certain proportion of zero-broomrape plants, even those coming from crosses between very susceptible lines. It is necessary to be as sure as possible that the selected plants are not escapees; the probability of this event will be smaller if its family is characterized by a high proportion of such plants. Of course, the comparison with the statistical distribution shown by susceptible checks is compulsory. For practical purposes, the analysis of the distribution of individual lines, which could be an impossible task if a great number of lines is handled, can be substituted by a double index characterizing each line: its proportion of plants with zero broomrapes and its average of broomrapes/plant.

3. Races of *Orobanche crenata*.

There is a very low level of host/parasite interaction. Host genotypes react almost uniformly to parasitic races, and viceversa (Cubero and Moreno, 1979; Hernández, 1987). New parasitic biotypes can originate, however, as *Orobanche crenata* populations are very heterogeneous chromosomically as well as genetically, interspecific crosses being also possible.

4. Breeding strategy.

Our original source of resistance was the line VF1071, obtained by continuous selection in a very infested field out of Giza 402, obtained in Egypt (Nassib *et al*, 1982). We followed a modified recurrent selection method which includes a progeny test within each one of the recurrent cycles to breed for resistance in faba beans. Use of insect-proof cages is required because of the partial allogamy of the host species, but yield tests have to be performed under open field conditions in a highly and homogeneously infested plot (the homogeneity of the plot infestation has to be periodically checked). Because of the statistical distribution of the parasite, mentioned above, selection between lines and/or segregating generations has been preferred to the simple average value of broomrapes/plant. Statistical designs should include many repetitions.

The steps followed are now summarised:

a) Selection has always been performed in a extremely highly infected plot; material under selection was kept in insect-proof cages; open field tests were also performed.

b) The best plants from the best lines, taking into account the within line distribution of the parasite, as mentioned above, were selected and selfed in glasshouse early in the following year to provide seeds for an usual second sowing under cage in the same year.

c) F2 and backcrosses in both directions provide the set of families for selection; because of the strong environmental effects on the broomrape attack, both generations are evaluated by progeny tests through respectively the F3 generations and selfed backcrosses. Although both backcrosses are very different to each other concerning the resistance level, lines must be selected from both backcrosses because, otherwise, lines derived from the backcross to the resistant parent will show more resistance but less adaptation.

d) As in any other recurrent scheme, it is essential to allow for a continuous recombination between the genes for resistance and the genes for yield. Thus, crosses between the best F2 or BC plants (identified by progeny tests as mentioned before) are performed; these crosses can be also performed directly between the best F3 and selfed BC.

e) Field trials with advanced materials are performed following a design based on a high number of repetitions, each one consisting in small individual plots including one advanced selected line surrounded by a susceptible check and the resistant parental.

The expected progress in selection will always be slow, as the characteristics involved (resistance, yield, seed size) are quantitative characters.

5. Genotype-environment interaction.

Eleven experimental lines of faba beans (including components of the cultivar "Baraca": Cubero *et al.*, 1992) were grown in 17 environments in Andalucía (southern Spain); the GxE interactions were studied by two multivariate methods (AMMI and Principal Component Analysis). Results of the stability analyses (Flores *et al.*, 1996) indicated that the most stable lines for resistance were not the same as those for yield. Three of these lines, L1, L2 (components of "Baraca") 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. Thus, progress in selection to accumulate both *Orobanche* resistance and yield is hampered by large environmental variation between locations. More breeding effort is still needed in order to improve the yield stability of the resistant lines.

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TABLE 1. Studies on resistance to *Orobanche crenata*

Method/Material	Results	References
Segregating generations Diallel 4x4	Expression of resistance depends of environmental conditions.	Cubero, 1973
Segregating generations, parasite populations	Resistance, Host-parasite interaction almost nil.	Cubero and Moreno, 1979
Diallel 6x6 F_2	Strong additive effects Very diff. genotypes No dominance. Scaling effect.	Cubero and Martínez, 1980
Diallel 8x8 F_1	Strong additive effects Weak partial dominance. Suscept. > resist.	Suso, 1980
Segregating generations Different genotypes	Additivity, No dominance Influence of the level of infestation.	Cubero and Hernández, 1991
Segregating generations Parasite populations	See Table 2. Expression of resistance depends of environmental conditions. Host-parasite interaction almost nil.	Hernández, 1987

TABLE 2. Genetics of resistance to *O. crenata*.
(Data from Cubero and Hernández, 1991)

Cross	Generations	Dominance (*)
170 x 172 S r	P_1 P_2 F_1 F_2 B_1 B_2 P_1 P_2 F_1 F_2 F3 (B_1) (B_2)	No yes S>R
1071 x 119 R VS	P_1 P_2 F_1 B_1 B_2 P_1 P_2 F_1 F_2 F_3 (B_1) (B_2)	No Yes S>R

(Follow in the next page)

Cross	Generations	Dominance (*)
1071 x 06 MS	P_1 P_2 F_1 F_2 B_1 B_2 P_1 P_2 F_1 F_2 F3 (B_1) (B_2)	yes S>R
1071 x 26 VS	P_1 P_2 F_1 F_2 BC_1 BC_2	S>R
172 x 06	P_1 P_2 F_1 F_2 B_1 B_2	yes S>R
172 X 119	P_1 P_2 F_1 B_1 B_2	yes R>S
172 x 26	P_1 P_2 F_1 F_2 B_1 B_2	No
119 x 26	P_1 P_2 B_1 B_2	Compl.
119 x 06	P_1 P_2 F_1 B_1 B_2	yes S>R
26 x 06	P_1 P_2 F_2 B_1 B_2	No

(*) Additivity was always significant, being the major component in all cases.

RESISTANCE TO *Orobanche:* GENETICS AND BREEDING

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Table 1 shows the number and proportion of papers published on general topics as well as on the specific item of resistance. In the period 1970-1998 there are 8-9 papers published yearly on research for resistance to *Orobanche* spp. If all the parasitic weeds are considered, the number is about 12 papers per year. During that period there were some 60 papers on *Striga* (50 on screening) and 8 on other parasitic weeds, *Cuscuta* alone being the subject of 6 of them (4 out of these 6 were on mechanisms of resistance).

The field that has registered the strongest increase in the last ten years (Cubero, 1991) is the study of mechanisms of resistance, which multiplied by a factor of 7 the figure recorded in 1988; at the present time accounts for a 15% of the total papers on resistance. Significant increases were also registered in methods, races and genetics, all of them doubling their former figures, accounting now for about 10%, 10% and 20% respectively of all papers on resistance. On the contrary, screening descended from almost two thirds of the total number of papers to less than 50%.

If the different *Orobanche* species are compared, the proportions are very similar to those obtained in 1988: about half of the mentioned papers refer to *O. cernua* on sunflower; as mentioned by Cubero (1991), had this survey included papers written before 1970 the situation would have been still more biased towards both *O. cernua*/sunflower and practical screening for resistance, as several papers on this subject were published by USSR breeders and agronomists (especially by Pustovoit and his colleagues) since 1910.

Useful levels of resistance have been found in several host/parasite systems as sunflower/ *O. cernua*, faba bean/ *O. crenata* and common vetch/ *O. crenata*.

Resistance has also been reported on eggplant/ *O. aegyptiaca* and several cucurbits/ *O. ramosa* and *O. aegyptiaca*. Observations on a world collection of peas also indicate the existence of a strong level of resistance. Chickpea breeders and agronomists also know that even under winter sowing, resistance does exist in breeders collections. Resistance is, henceforth, the most hopeful field of research, even though its handling by the breeders is not easy at all.

TABLE 1. Publications on resistance to *Orobanche* species

	total number	cernua	crenata	ramosa	aegyptiaca	minor
			percentages (rounded figures)			
On any topic (1935-1998)	1170	17	14	18	10	5
On resistance				(percentages on n=260)		
	260	42	29	11	14	
** screening	115	22	12	6	4	-
** methods	28	4	4	1	2	-
** races	25	7	1	1	0	-
** genetics	25	8	9	1	3	-
** mechanisms	39	5	3	2	5	-

Sources: Surveys on CAB, BIOL and AGRO databases, on WRO Annotated Bibliographies, on Plant Breeding Abstracts, on Proceedings of International Conferences as well as on AGRIS and MEDLINE databases.

Genetics

Orobanche cernua/cumana // sunflower *(Helianthus annuus).* First results by Pustovoit indicate the presence of a polygenic system mostly showing no dominance (Pustovoit, 1966). A single mendelian locus with two alleles, resistance being dominant, was described later on by Pustovoit (Kolte, 1985). A monofactorial type of inheritance was also described by many other authors. The appearance of *Orobanche cernua* races complicated the inheritance pattern; thus, either complementary genes (Krokhin, 1980) or a system of four matching genes (Vranceanu *et al.*, 1981, 1987) were suggested.

Orobanche crenata/ faba bean *(Vicia faba)*. A revision can be seen in this volume.

Orobanche crenata/ common vetch *(Vicia sativa)*. Gil *et al.* (1982, 1984, 1987) found a system very similar to that found in faba bean: an almost purely additive system with very weak dominance, if present; the environmental conditions also were important.

O. aegyptiaca/ tomato *(Lycopersicon esculentum)*. Resistance of tomato seems to show dominance or overdominance, the results showing the presence of 2-3 major genes and 2-4 minor genes (Avdeev and Shcherbinin, 1976).

O.ramosa/ tobacco *(Nicotiana tabacum)*. Vinogradov *et al.* (1981) found tolerance and suggested that it was controlled by 1-2 pairs of recessives and some modifier genes.

Mechanisms of resistance.

Anatomical mechanisms.

Resistance of sunflower to *O. cernua* was produced, according to Pustovoit (1966) by the formation of callus originated in swellings in the root and the accumulation of lignin-like compounds in damaged root cells (Antonova, 1978).

Giza 402 resistance in faba beans to *O. crenata* was attributed to a larger thickness of the root cortex (Nassib *et al.*, 1982), but no relationship between root cortex and resistance could be seen in the roots of F2 plants from crosses between VF1071 (derived from Giza 402) and susceptible lines (unpublished results). Recent observations suggest a dynamic, hence biochemical, response of the host against the parasitic attack; the possible structural changes could be just a consequence.

O. aegyptiaca haustoria form tracheids that can penetrate the tomato root, as *O. ramosa* on pumpkins, cucumbers and melons, according to Kabulov and Kabulova (1977).

Biochemical mechanisms.

Differences involving enzymatic activities, protein fractions and inmunological reactions can explain differences between resistant and susceptible *O. cernua* genotypes (Avramenko, 1973; Lyalyushkin et al., 1975). Resistance to *O. aegyptiaca* could be related to the tomato transpiration rate (Faizieva, 1978), the parasitized susceptible lines showing a more intense fall in transpiration rate as well as in photosinthetic activity than resistant ones.

Differences between root exudates from resistant and susceptible genotypes of tobacco to *O. ramosa* have also been found (Racovita, 1973). But these facts not yet provide an explanation for resistance.

Breeding methods.

O.cernua/cumana // sunflower. Pustovoit used individual selection with progeny test. Later on, recurrent selection schemes were followed until the present. Pustovoit used two indexes of resistance: the "affection level" (percentage of plants attacked by the parasite) and the "degree of affection" (number of broomrapes per host plant; Pustovoit, 1976; Pustovoit and Khatiyanskii, 1985, inter alia) with or without incorporating selfing in the process. Interspecific hybrids with *(Helianthus tuberosus)* have been also used (Pustovoit,1966; Kostiuk, 1986, and others) as well as with *H. petiolaris* (Vranceanu *et al.*, 1980) and wild North American sunflowers (Pustovoit and Skhuropat, 1978; Pustovoit and Khatiyanskii, 1980). Some breeders prefer individual selection under strong artificial field infestation (Khatnayanskii, 1988).

*O.crenata/*faba bean. See the review in this volume.

O.crenata/ common vetch. Individual plant selection in a large collection provided many resistant lines. Resistance was absolute in some cases both under field and greenhouse conditions. The number of broomrapes per host plant was more stable than both the weight and height of parasitic plants per host plant (Gil *et al.*, 1982, 1984, 1987).

O. crenata/ lentil. A good laboratory screening method does exist (Sauerborn *et al.*, 1987) but only differential levels of susceptibility have been found until now.

O. crenata/pea. Promising sources have been identified in a very large germplasm collection, including wild and relatives and primitive races, and several crosses have been performed both to study the genetics of the resistance and to transfer it to commercially valuable lines (see the review in this volume).

O. ramosa/tobacco. Only field tolerance has been found even by using large collections of tobacco, including wild species and chemically induced mutants (Vinogradov *et al.*, 1981; Palakarcheva and Voinova, 1986), but resistant lines have been reported derived from *N. tabacum x N. goodspeedii* crosses (Palakarcheva *et al.*, 1987) and from mutants obtained after treatment with N-nitroso-N-ethyl urea and ethylenimide.

O. aegyptiaca and *O. cernua*/tomato. Only tolerance and moderate levels of resistance to both species were found on rather small germplasm tomato collections. *Lycopersicon hirsutum* and *L. esculentum pyriforme* and *L. e. pruniforme* have been mentioned as reliable sources of resistance (Avdeev and Shcherbinin, 1983).

O. cernua /eggplant. A few highly resistant eggplant lines have been reported.

O. aegyptiaca/ cucurbits. Many reports point to failure or very scarce success in finding resistant materials in *Cucurbita pepo, Cucumis melo, Cucumis sativus* and *Citrullus lanatus* (Mukumov, 1970; Mukumov and Faizieva, 1977). The index used was the number of parasites per host plant, either emerged or attached to the the roots of the host.

Orobanche ramosa/ hemp *(Cannabis sativus).* As in many other cases, only tolerance or moderate levels of resistance have been found.

O. aegyptiaca/ rapeseed *(Brassica campestris)* and mustard *(B. juncea).* In small germplasm collections, some resistant genotypes have been identified in both hosts by Dalela and Mathur (1971) but none by Sobrino (1985) in rapeseed.

Pathogenic biotypes

O. cernua/cumana // sunflower. Biotypes of *O. cernua* (namely races A and B) were identified very soon, differing not only in the pathogenic reaction but in biology, physiology and even morphology (Pustovoit, 1966). New races were described in other countries when the new cultivars spread out of the former USSR (Vranceanu *et*

al. 1981; Gonzalez-Torres, 1982). New dangerous races have recently appeared in Spain and Turkey, challenging sunflower cultivation in important areas if these races spread out of their locations.

Orobanche crenata/ faba bean. See the review in this volume.

Orobanche ramosa and *O. aegyptiaca*/ tobacco. Differences in virulence among races were found in both parasites under greenhouse conditions (Vinogradov *et al.*, 1981, 1983).

Orobanche ramosa/ hemp. Resistant varieties were obtained in France during the XIX century, but they were wiped out later on by the same parasite suggesting that the latter was able to build up new genic combinations for virulence.

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BROOMRAPE RESISTANCE IN FABA BEAN: WHAT DO WE KNOW ?

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Introduction.

In this contribution I will review information about resistance against *Orobanche crenata* Forsk. in faba bean (*Vicia faba* L.). Resistance in its strict sense ('true resistance') indicates processes in the host root which prevent establishment of the parasite. Usually it is quantified as the number of spikes per host plant, but a more precise parameter in fact is the number of established parasites per unit host root length. However, plants may have also other characters reducing negative effects of the parasite on crop yield, namely avoidance or tolerance. In case of avoidance, the interaction between host and parasite is prevented in some way, other than true resistance. A tolerant host is able to support the parasite and itself, resulting in good growth of both. Resistance in its broad sense covers these three mechanisms, including all cases where crop yield is little or not reduced in spite of the presence of germinable broomrape seeds in the host root environment.

In this review I will present studies comparing more or less resistant and susceptible faba bean material, discussing plant characters that possibly add to resistance. The review concentrates on studies comparing faba bean variety Giza 402 and its relatives with susceptible cultivars. For reasons of comparison some additional work on sunflower is included.

The genetic basis of resistance.

In 1997 Lane *et al.* published a paper on the genetic basis of plant-parasite interactions, discussing relations between *Striga* spp. and their host crops in Africa, between *Orobanche cumana* Wallr. (syn. *O. cernua* Loefl., Pujadas, this volume) and sunflower, and between *O. crenata* and faba bean. Whereas resistance in the first two

groups is based on gene-for-gene relationships, resistance in faba bean is polygenic and additive, which makes its breading more complicated (see e.g. Cubero 1994; Cubero *et al.* 1994). This is illustrated by the differences in resistance breeding between faba bean and sunflower.

Resistance in faba bean is based on a cross between the commercial Rebaya 40 and the land race F216, collected in the seventies in Upper Egypt; in this region faba bean had been grown since long in broomrape infested soils. It resulted in F 402, and later in the resistant cultivar Giza 402, produced for the Egyptian market (Nassib *et al.* 1978; Khalil *et al.* 1994; Khalil, this volume). These genotypes were the basis for the faba bean races and cultivars with true resistance developed later on. On the other hand, sunflower only became subject to broomrape attack after its introduction into Europe in the 19th century (Sackston 1992). It originated from the American continent, where it had not been in close contact with broomrape. Since the beginning of this century, Russian breeders, with their colleagues in other countries, have produced a series of new cultivars, each adapted to newly developed more aggressive broomrape populations (Pustovoit 1976; Dominguez, this volume). In fact, one might state that sunflower breeders are doing now what faba bean farmers had done long ago: selecting, may be breeding, resistant material.

The mechanistic basis of resistance.

The shoot

Stimulus of host growth

When analysing plant growth (including root production) over time, we noticed in several experiments, that at an early stage of development, before pod production starts, the infested faba bean host + parasite, even the host alone, had a higher dry weight than the non-infested one; the phenomenon was only observed when growing conditions were optimal (water, fertilizers). The effect was first noticed in greenhouse experiments, but appeared to occur in field experiments as well (Ter Borg and van Ast 1991a; Mesa-García and García-Torres 1986). Apparently the faba bean system for carbohydrate production has a certain over-capacity, which is becoming active only when a 'sink' is available. It results in -at least temporary- tolerance of the host, allowing full growth of the host and the parasite. The effect was more obvious in Giza 402 than in other cultivars (Fig. 1)

Various mechanisms may be at the basis of this phenomenon, such as a longer leaf life span, formation of extra tillers or increased photosynthesis (cf. Press, this volu-

me). This last option may be related to the higher concentration of peroxidase in the leaves (and roots) of infested faba bean, reported by Kirollos and El-Hafeez (1985).

The tolerance reported is only temporary. It is lost in later stages of growth, and according to Manschadi broomrapes and growing pods are then competing for the carbohydrates produced. Final dry weight of host plus parasite is similar to the uninfested control (Manschadi, this volume), which implies a balance between the production of broomrapes and pods. Patterns observed were similar in both, the susceptible ILB 1814 and the resistant G402/29/84.

It is not clear whether this mechanism of partial/temporary tolerance is linked to true resistance, and therefore is present in all other resistant cultivars as well. It can be questioned also, whether the stimulus on growth can be found in all host species, or just in those having the specific capacity required (cf. Press, this volume). Anyhow, when testing this phenomenon, growing conditions of the host should be optimal.

Date of first flowering and sowing date.

Establishment of the parasite is only possible after a host root system has developed, and broomrape seeds have been stimulated to germinate. Therefore, broomrape growth starts later than that of faba bean. This implies that early flowering hosts will suffer relatively less from broomrape attack than the later ones, since their first pods may have developed before broomrapes start to compete for minerals and carbohydrates. The moment of first flower production is correlated with the number of nodes on the main stem below the first flower (Ter Borg, unpubl. data). Since this number can be recorded at any time after flowering has started, earlyness can be quantified by counting node numbers at harvest. Therefore, this way of avoiding competition is open for selection and breeding.

The above will only apply if broomrape development is independent of host flowering. However, some authors indicate that there is some relation between flower production and spike emergence, e.g. Ghobashy (1997). This author worked with the resistant lines Cairo 241 and Cairo 348 that have resulted from the breeding programme of Cairo University (Abdalla and Darwish, 1994).

When grown as a winter crop, delayed sowing may result in higher faba bean yield, due to reduced broomrape infestation. The method works as long as the lower temperatures of later sowing dates do not reduce crop growth. The effect may be

due to a direct influence of the lower temperatures at later dates on germination percentages, or on broomrape seed dormancy in autumn and winter, or both (Van Hezewijk 1994). Sowing date also has a different effect on the development of parasite and host, and so affects the timing of pod production and broomrape growth (Ter Borg 1987; Fig. 2). This may have consequences for their interaction.

For a further analysis of growth and development of parasite and various host cultivars simulations will be helpful. Some models have already been developed (Kropf and Schippers 1986; Ter Borg et al. 1994 a; Manschadi 1997; this volume). They can be used to study in more detail the effects of sowing date, date of first flowering and related plant variables under a range of climatic conditions. The results can be used to predict the effectivity of breeding for earlyness.

The root.

Root mass

Often broomrape vigour is related to host performance. Therefore, low parasite numbers may result from a small root system, either by bad host growth or by a higher shoot/root ratio (Khalaf and El-Bastawesy 1989). In both cases, wrong conclusions about 'resistance' are nearby. Successive selection of material with low broomrape numbers may result in avoidance in hosts with a small root system; this, however, is a risky character in regions with low rainfall (Aalders and Pieters 1986).

Root architecture.

More than root mass, distribution of roots with depth, below the soil layers containing most broomrape seeds, might help to avoid broomrape attack. With respect to Giza 402 Nassib *et al.* (1978) stated that its 'resistance is associated with slower tap root growth, less production of lateral roots and an altogether more compact root mass'. Deeper rooting of this cultivar was noticed by Pieterse (unpubl. data 198?) when comparing Giza 402 with some susceptible material grown in deep vessels filled with vermiculite.

For a more detailed study of root architecture we planted Giza 402 and Giza 2 under dry and moist conditions in large, ca 40 cm deep boxes with openings, allowing quantification of root development over time (Ter Borg and Van Ast 1991b). The data recorded included root length, numbers of root tips and root distribution. Most roots and *Orobanche* attachments were found in the upper 15 cm of soil, with con-

tinuous root growth and broomrape establishment on young roots in all layers. Numbers of established broomrapes per unit root length were highest under moist conditions, and were lowest in Giza 402 (Figure 3). There were some indications that lateral roots of Giza 402 were at slightly lower positions, and that the maximum depth of its roots might be somewhat more than in Giza 2, but the difference was minimal and could hardly have any significant influence.

Stimulant production.

Low stimulant production by host roots might prevent germination, and hence establishment of the parasites, as already suggested by Nassib *et al.* (1978). It was found to reduce *Striga* attack in some sorghum cultivars (Ramaiah 1987). So far, neither in resistant faba bean (Khalaf and El-Bastawesy 1989; Van Woerden *et al.* 1994) nor in sunflower (Dörr *et al.* 1994) low stimulant production was found to play a role as a mechanism of avoidance. On the contrary, the activity of germination stimulants produced by resistant host material appeared to be at least as high as that of the susceptible ones, or even higher.

Resistance s.s.

Nassib *et al.* (1978) stated that resistance in F 402 might be due to 'the erection of mechanical and physiological barriers to the sucessful establishment of the parasite'. Khalaf and El-Bastawesy (1989) probably were the first to publish a more detailed anatomical study, comparing a.o. the anatomy of lateral roots of - probably uninfested- Giza 402 and the susceptible cultivar Aquadulce. Amongst other things they reported a slightly thicker epidermis and cortex in Giza 402. Attia (1992) recorded less secondary growth of the vascular tissue, later rupture of the endodermis, absence of fibrous cells with cellulosic cell walls, and lignification of the secondary xylem. She also noticed increased cell growth at the site of attack. This reaction might be the anatomical basis of the thickening and wart formation which was sometimes observed in faba bean, as e.g. by reported by Ter Borg *et al.* (1994b). These authors noticed warts on secondary roots of faba bean, in the resistant line 402/294 in particular, when comparing a set of nine resistant and susceptible cultivars. Earlier, Masri (198?) related such warts to *Rhizobium* nodules. However, later it was found that similar structures develop on roots of faba bean mutants lacking the capacity to form root nodules (Cubero, Khalil, pers. comm.). Moreover, similar warts were noticed on roots of sunflower (Petzoldt, pers.comm.). Hence, their development must be independent from root nodule production.

According to Zaitoun *et al.* (1991) who studied host root anatomy of infested plants, a corky tissue develops in Giza 402 as a hindrance to broomrape penetration. The few haustoria present were thin and pinshaped, in strong contrast to the thicker ones growing on the susceptible cultivars Giza 3 and Reina Blanca. In a morphological study Zaitoun distinguished three structural patterns in resistance, necrosis of host root cells, either just before or after broomrape had penetrated into the host root, and development of a barrier after formation of a small tubercle. Essentially the same phenomena were noticed in the resistant Egyptian lines 402/29 and 674/154 and resistant Baraca from Spain (Zaitoun and Ter Borg 1994). This confirmed that resistance was brought from Egypt to Spain, due to the Spanish breeding activities (Cubero 1994; Cubero *et al.*1994).

The morphological and anatomical patterns distinguished must have a physiological basis. Increased peroxidase in infested faba bean roots reported by Kirollos and El-Hafeez (1985) might be the basis of the thickening of the root cortex, maybe also of wart formation. Various authors hypothesized about a possible role of phytoalexins in relation to broomrape resistance (Wegmann 1994; Alonso 1999 and this volume; Jorrin *et al.* 1999 and this volume). Information on the role of exo- and endoenzymes during broomrape penetration came from Israeli workers (Joel 1999 and this volume). However, these authors worked with other species combinations.

Various structures in resistant faba bean were described in reasonable detail; yet little order or interrelation between them was suggested so far. The situation is different with respect to sunflower and *O. cumana*, where various mechanisms in host and parasite were distinguished (Ukrainskiy 1938, Panchenko and Antonova 1974, 1976; Antonova 1978, 1994). Based on these earlier observations Antonova and Ter Borg (1996) proposed an order between the various phenomena, with a central role of peroxidase production by the parasite. In the host they distinguished swelling of the host root resulting in wart formation, thickening of cell walls, hypersensitivity and necrosis, production of phenolic compounds, and lignin formation between the young haustorium and adjacent xylem vessels. Broomrape populations differed with respect to rate of peroxidase production and its localization. Each of these characters was related to a specific gene in the sunflower host or to one in the broomrape races. The work covered sunflower genes Or1 through Or3 and *O. cumana* races A through D, as described in Russia. It still has to be awaited whether the genes and races recorded under the same letters and numbers in various countries in southern and eastern Europe always have the same biological basis. Moreover, it is not yet clear in which way the Russian work can be related to other studies, e.g. that of Dörr *et al.* (1994) who found slow growth of broomrape in the cortex and lignification of cell walls in sunflower cul-

tivar 'Edirne'. More intraspecific variation has been described meanwhile, with up to gene Or6 for resistance in sunflower and race F in *O. cumana* (Dominguez, this volume). The new ones have not yet been related to specific processes; Alonso (1999 and this volume) mentioned the possible role of phytoalexins in this context.

Conclusions.

When comparing the anatomical structures resulting in true resistance, at first sight, many phenomena are similar in sunflower and faba bean. They share this similarity with other plants attacked by a parasitic plant (Sallé *et al.* 1991; Lane *et al.* 1997; Goldwasser *et al.* 1997). The hypersensitive response is a very common phenomenon that has been observed in plants attacked by a wide range of pathogens, including viruses, bacteria, fungi, nematodes and insects (de Wit 1997).

In contrast to the suggested similarity of the mechanistic basis of resistance, the genetic basis is essentially different. In sunflower the interaction between host and parasite seems based on a gene-for-gene system, and resembles to some extent the elicitor-receptor systems, as described for plant-fungal relations (e.g. Laugé and de Wit 1998). The basis of faba bean resistance is a set of polygenic and additive characters. The set seems to be fairly closely linked, as it was bred as a package into new cultivars far outside its origin in Upper Egypt (Cubero 1994, Cubero et al. 1994). Evidently, several factors must contribute to build an effective barrier. However, various types of barriers may be independent, and be passed separately during the breeding process, as e.g. a true barrier formed by a cork layer or lignified cell walls as against cortex thickening leading to wart formation. Their combination might be a good basis for rather strong resistance, as e.g. seems to be the case in Giza 402/294, tested by Ter Borg *et al.* (1994b).

Independent inheritance is also to be expected for factors for tolerance and avoidance. Four of these were discussed in more detail. Effectivity of the temporary tolerance described seems to be limited. The same holds for root architecture leading to avoidance, the more so because of the high plasticity of root systems and variability of soil conditions in a field situation. Avoidance based on low stimulant production may be effective, as seen in the *Striga*-sorghum system, but it will require a lot of work to find and select this factor, if it exists at all, in faba bean.

Avoidance of competition between broomrape and pod development by early flowering seems to be an effective independent characteristic. Apparently it has been

bred into line X-843, with true resistance and early maturity (Saber *et al.* 1999); the same may hold to some extent for line 402/294 (Ter Borg *et al.* 1994). Simulation seems to be the appropriate method for a further analysis of the effects of these factors and their interaction with climatic variables. The analysis could also include the effects of temporary tolerance. Simulation results can then be used to direct future breeding programmes.

The mechanistic basis of resistance factors in faba bean is complex, but must basically be due to separate genes coding for various biochemical processes, probably organised in some sets of closely linked genes. At least part of the reactions must be elicited by the parasitic attack. So far, hardly any information is available about genetic variation in *O. crenata* and its possible role in the selection and evolutionary history of faba bean resistance. Preliminary data from Ghobashy (1997) on different virulence of two populations of *O. crenata* against *V. faba*, and information from Joel (this volume) on the increased aggressiveness of faba bean 'race A' against *V. sativa* suggests that relevant variation does exist. This indicates that a gene-for-gene system, albeit far more complex, might regulate at least part of the interaction of faba bean with broomrape, as it does in sunflower.

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Table 1. A prelimnary scheme for relations between the mechanisms of O. cumana virulence and sunflower resistance genes

O. CUMANA		SUNFLOWER		
races	characteristics	genes	characteristics	resistant cultivars in Russia
		Or 0	–	No resistant cultivars until 1916
race A	'Wildtype' on *Artemisia* spp. and local sunflowers. Slow growth into host	Or 1	Swelling on host root at site of intruding parasite; haustoria die in its centre	Kruglik 7-15 163 Saratovskii 16 Kruglik 631 Kruglik A-41
race B	Double amount of peroxidase in stem	Or 2	No swelling of host root. Dense cell walls underneath epidermis. Hypersensitivity and necrosis in cortex, small amount of phenolics compounds	Jdanovskii 8281 Jdanovskii 6432 VNIIMK 8931 Peredovik Moldavskii 41 Armavirskii 3497 and other (Edirne?)

(Follow in the next page)

O. CUMANA		SUNFLOWER		
races	characteristics	genes	characteristics	resistant cultivars in Russia
race C	High speed of growth into host; intra- and extracellular peroxidase production	Or 3	Increased production of phenolics compounds; lignin precursors in cortex. Lignin formation at site of contact between young haustorium and xylem vessels	October Progress Peredovik Or/R Yubeleinii 60 Beresanskii and others
race D	Peroxidase not secreted outside apical cells; intracellular peroxidase only	Or 4	??	So far no cultivars developed in Russia (but see Shindrova, 1994, and Encheva & Shindrova, 1994)
race E	??	Or 5	??	see under Or 4

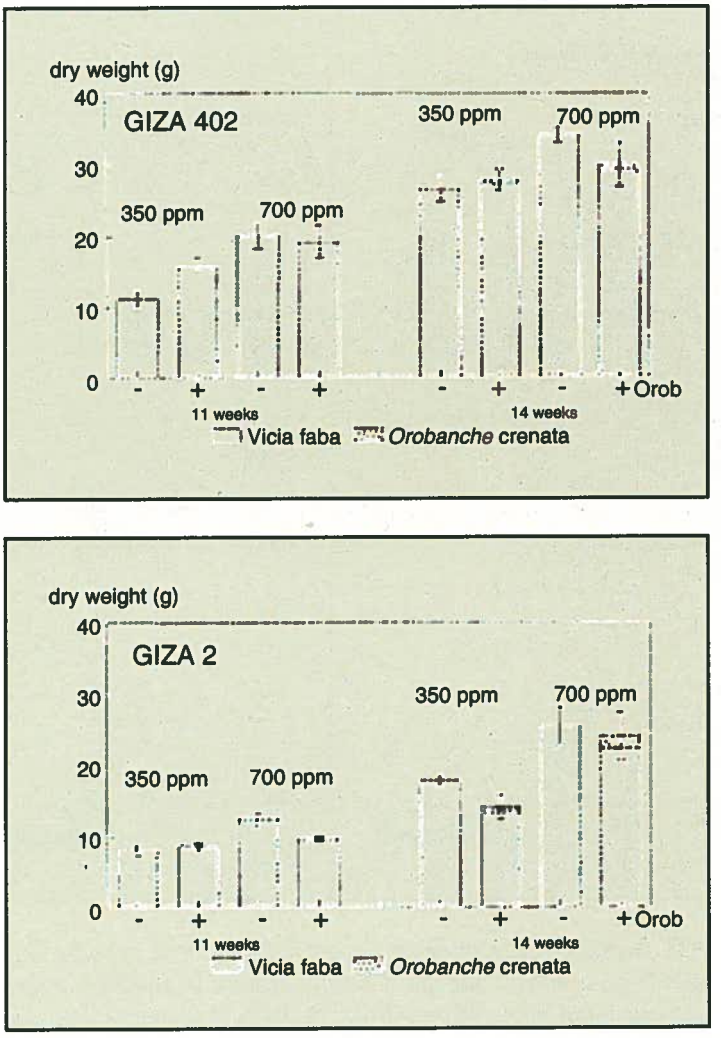


Figure 1: Growth stimulus after 11 weeks, at ambient CO_2, in Giza 402, not in Giza 2. Average values + s.e. (From Ter Borg and Van Ast 1991a).

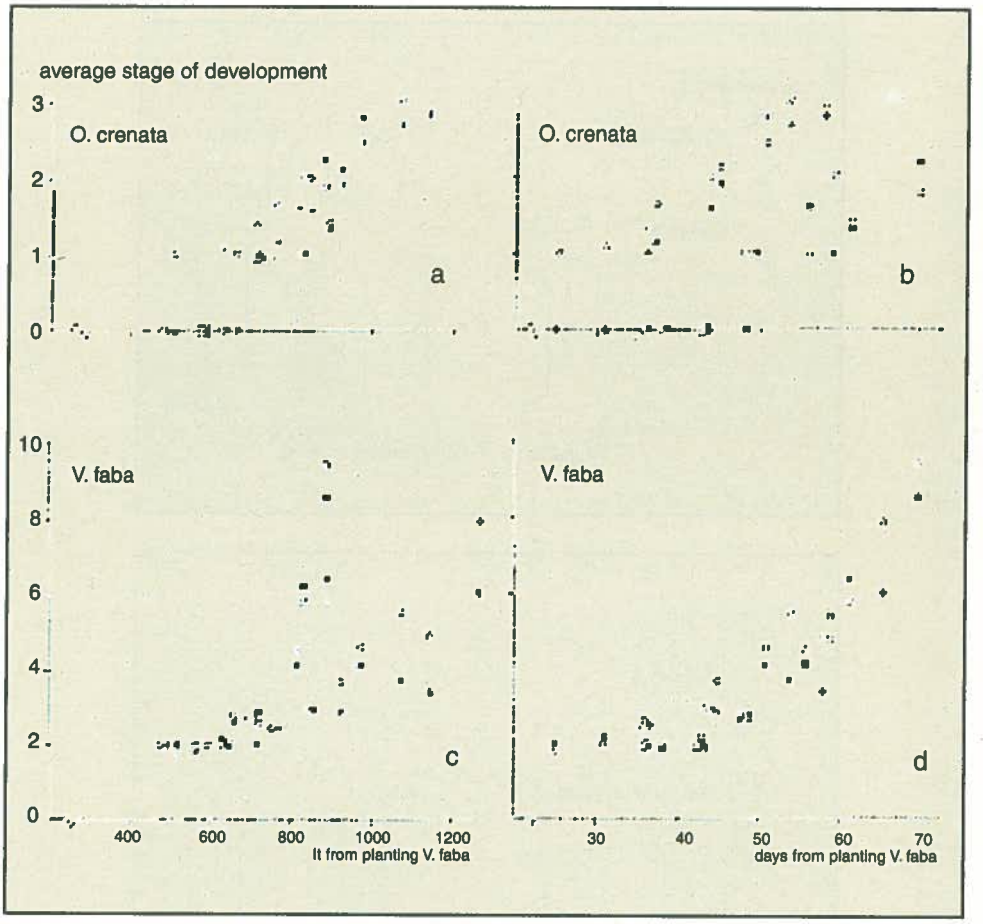
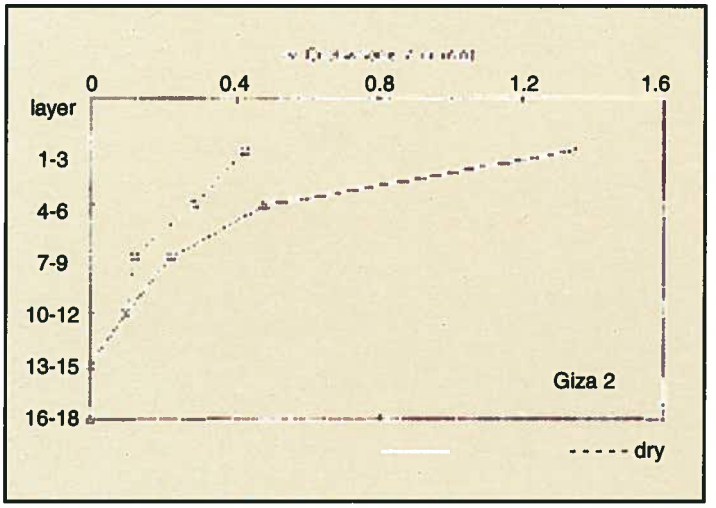


Figure 2: Average stage of development in *Orobanche crenata* (Fig. 2a and b) and *Vicia faba* (Fig.2c and d) against sumtemperature (cumulative average of dally maximum and minimum temperatures from the date of planting) (Fig. 2a and c) and against time (number of days after planting) (Fig.2b and d). Note that development of *O. crenata* correlates with sumtemperature, and that of *V. faba* with time.

Planting dates faba bean: Giza 2: 13 October O and 10 December □, Giza 402: 27 October • and 23 December ■ (From Ter Borg 1987).



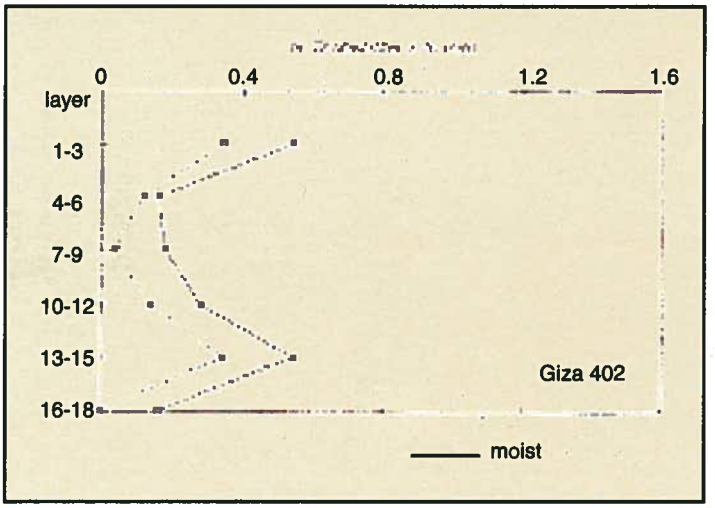


Figure 3: Number of attachments per meter root length in Giza 2 and Giza 402 under moist and dry conditions. (From Ter Borg and Van Ast 1991b).

RESISTANCE IN Vicia sativa L. TO Orobanche crenata FORSK.

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Introduccion.

Vicia sativa is a grain legume crop growing mainly in the Mediterranean Basin where its seeds are used principally for animal feed; it is also used as a forage plant. In Spain are sown about 250.000 ha for grain and 100.000 ha for forage (MAPA, 1997).

Vetches have been mentioned as hosts of *Orobanche crenata* but this parasitic plant has not been a serious problem in this crop as it is the case in other grain legumes (faba bean, pea and lentil). In the 70's we started a breeding program to obtain resistant varieties. Field observations showed both a high variability in this species in its response to the attack of *O. crenata* and a high level of resistance. Three good yields resistant cultivars were released (Martín *et al.*, 1982).

Characterisation of resistance.

The resistance of the accessions was confirmed in natural infested fields at two locations. Fourteen resistant and susceptible accessions were sown following a randomised complete block design in each location. In both locations a clear resistance was confirmed in the field (Gil *et al.*, 1982).

In greenhouse, an experiment with two resistant and two susceptible lines sown in inoculated pots with two doses of broomrape seeds (17 and 83 mg per kg of sand) was performed. The attack of broomrape began at the same time in all lines. Differences between resistant and susceptible genotypes were found for the number of broomrapes per plant (attached on the root) with higher differences for the high dose where was possible to differentiate between resistant lines. About the development of the broomrape along the time no differences were found between resistant and susceptible lines (Gil et al., 1987). According to these results, the resistance seems to be due to a lewer number of broomrapes growing on resistant plants than on the susceptible plants.

Genetics of Resistance.

Quantitative inheritance for resistance of vetch to broomrape was found (Gil *et al.*, 1987). The genetics of resistance was studied using F1, F2, F3 and backcrosses generations from four crosses between resistant and susceptible lines growing in a infested field during two years. The attacks (measure as broomrapes per plant) was higher in the second than in the first year. It could be explained by different climatic conditions during these years. This fact could explain the different results obtained in this study; in the first year additive and dominant genetic effects were significant; only additive effects were detected in the second year (Gil *et al.*, 1987).

The level of resistance found in *Vicia sativa* is much higher than that found so far in *Vicia faba*. The quantitative expression of the resistance in the field may be due in part to the environmental effects, as climatic conditions or level of homogeneity of the broomrape seeds in the soil.

At the present time, a new methodology to study the resistance to *Orobanche* in controlled environmental conditions has been developed in *Lens*. In our breeding group, this methodology is being applied to other legumes including vetches. The studies of the genetic of the resistance under these controlled conditions could give us a more precise knowledge about its inheritance and a more effective searching of markers associated to the resistance.

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NEW SOURCES OF RESISTANCE TO BROOMRAPE (*Orobanche crenata*) IN A COLLECTION OF VICIA SPECIES.

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Introduction.

Orobanche crenata Forsk. is a holoparasitic weed that seriously attack legume crops, such as faba bean, lentils, peas, chickpea and vetch, but also a large number of wild legume species (Cubero, 1983). The broomrape problem has been common to the farmers from the old times. The extraordinary high number of seeds and their high viability in the soil difficult the control of this parasitic weed. Although different control methods have been proposed to avoid this serious problem, the most successful recommended practice was crops rotation with low frequency of susceptible crops (Mesa-García and García-Torres, 1991), which would mean a considerable reduction in the growing of legumes, what is not desirable in the frame of a Sustainable Agriculture. So, the development of resistant cultivars is a major need. Good levels of resistance to *O. crenata* have been found in faba bean (Nassib *et al.*, 1982; Cubero, 1991; Sillero *et al.*, 1996a), common vetch (Gil *et al.*, 1987), wild *Vicia* species (Linke *et al.*, 1993; Sillero *et al.*, 1996b) cultivated and wild peas (Rubiales et al., 1998) and chickpea (Rubiales *et al.*, this volume). Some purple vetch (*V. atropurpurea*) genotypes have also been described as resistant to *O. aegyptiaca* (Goldwasser *et al.*, 1997).

The objectives of the present work were to identify new sources of resistance to *O. crenata* in a *Vicia* spp. collection; to confirm this resistance in pot experiments; and to study the resistance mechanisms in vitro.

Materials and Methods.

Two hundred and ten accessions belonging to 42 *Vicia* species were screened for resistance to broomrape under field conditions during the growing season 1995/96. These accessions came from different origins, all around the world. Each line was sown in a 1m row, surrounded by four rows of the faba bean susceptible check cv. Prothabon. The sowing took place late November, at Córdoba (Spain), in a field heavily infested with *O. crenata*. Hand weeding was done when required, but no herbicides were applied. At the end of the growing season, late May, the final number of emerged and non-emerged broomrape shoots per plant was counted and expressed as a percentage of the mean of its four surrounding rows of Prothabon (=100 %). 41 lines without emerged and with few emerged broomrapes were selected and studied in the infested field two more years (1996/97 and 1997/98). The field designs and the broomrape evaluations were similar to that of the first year.

The thirty-one most promising accessions were also studied in a pot experiment in a growth chamber. The faba bean variety Prothabon and the pea variety Messire were included as susceptible checks. Five days old plants were translated to 1 litre plastic pots filled with vermiculite, previously mixed with 25 mg of broomrape seeds (about 8000 seeds). Each genotype was represented by 12 plants, 1 plant/pot. 90 to 120 days after sowing, when the plants were mature, the plants were extracted, the roots were washed in water and the number of broomrapes was counted. The broomrape attachments were classified according their stage of development, following the 0 to 7 scale of Ter Borg *et al.* (1994).

The germination of broomrape seeds and the first steps of the attachment in nine resistant lines were studied in a petri dishes experiment, using the procedure described by Sauerborn *et al.* (1987). The pea variety Messire was used as susceptible check. One month after transplanting the *Vicia* spp. seedlings, 500 seeds per dish were studied and classified to determine the percentages of germination and establishment (established nodules of the total germinated seeds that contacted the roots).

Results.

On the basis of the first year data (Table 1), we found high levels of resistance in all the studied lines of the species *V. amphicarpa, V. cracca* var. *cracca, V. cuspidata, V. dalmatica, V. disperma, V. grandiflora, V. hirsuta, V. hyrcanica, V. incisae-*

formis, V. megalotropis, V. melanops, V. michaxii, V. onbrychioides, V. orobus, V. peregrina, V. tennifolia, V. tetrasperma and *V. vicioides*. We also have found intraespecific variation in resistance to *O. crenata* in some species, such as *V. articulata, V. bithynica, V. cordata, V. hybrida, V. monantha, V. narbonensis, V. pannonica, V. sativa, V. serratifolia* and *V. villosa*. In these species we found from very resistant to susceptible or highly susceptible accessions.

The most promising lines were studied in the field two more years, to confirm their resistance. Although the weather conditions both years were particularly conductive for the broomrape development and emergence, all the studied lines showed low levels of emerged broomrapes (Figs. 1 & 2), with less than 40% of the susceptible check. Only with few exceptions, these lines also showed low number of underground broomrapes.

In the pot experiment (Table 2) the different developmental stages of the broomrape could be studied. Twenty two accessions had less than 2 broomrape tubercules per plant, which means less than 13% of the susceptible faba bean and pea checks. In almost all the studied lines, the broomrapes presented early developmental stages. Seventeen accessions showed very low amount of broomrape buds (lees than 0,5 buds per plant) and in ten more accessions the maximum development of the broomrapes was a crow of roots, but no bud was developed. So even when some establishment was made there was restriction to broomrape development, which could be considered a mechanism of resistance. When the pots and the three years field data are analysed together, 21 lines can be described as new sources of resistance to *O. crenata*.

In petri dishes, other different operative mechanisms of resistance could be detected (Table 3). All the studied lines showed less number of establishment than the susceptible check. Different clear mechanisms of resistance have been detected. One of them is reflected as a lower percentage of broomrape seeds germination. It was present in all the lines. Other mechanism become apparent in some lines (V-52, V-133 and V-193), as a resistance to the establishment of the broomrape into the plant root system. In some rare cases, the *Vicia* root developed necrotic lesions surrounding the contact points of the parasitic radicle, which could be consider the last mechanism of resistance, although we did not see it consistently.

Discussion.

In the present work new sources of resistance to *O. crenata* have been described in the genus *Vicia*, belonging to species different than the cultivated *V. faba*. We have found good resistant levels in species such as *V. sativa*, the cultivated common vetch, and *V. villosa*, both traditionally cultivated as fodder crop (Guerrero, 1983). We have found intraespecific variation in resistance to *O. crenata* in some species, such as *V. articulata, V. bithynica, V. cordata, V. hybrida, V. monantha, V. narbonensis, V. pannonica, V: serratifolia, V. sativa* and *V. villosa*. The intraespecific variation in resistance in *Vicia* has also been described by Linke *et al.* (1993).

One of the recommended ways to reduce the broomrape seed bank is the use of host plants as catch crops, but they have to be harvested before the shoots emergency. Other control measure is the use of trap crops. These are resistant plants that stimulate the germination of *O. crenata* seeds, but avoid the establishment. In this sense, non legume crops, such as flax (Cubero, 1983), basil and coriander (Schnell *et al.*, 1994), as well as the legumes *V. villosa* ssp. *dasycarpa* and *V. narbonensis* (Saxena et al., 1994) have been successfully used and the seeds bank of broomrape have been notably reduced. All the studied accessions of some species, such as *V. cracca var. cracca, V. dalmatica, V. disperma, V. grandiflora, V. hirsuta, V. hyrcanica, V. melanops, V. michaxii, V. palaestina, V. peregrina* and *V. tennifolia*, have been resistant to *O. crenata*. The accessions of these species studied in petri dishes induced less germination of the broomrape seeds than the susceptible check, but might still be useful to reduce the seed bank in the soil.

Resistance to establishment of the tubercule is clear in accessions V-52 of *V. hirsuta*, V-133 of *V. villosa* and V-193 of *V. tennifolia*. We can not say at what stage that resistance is operating. It could act from a physical barrier to host tissue penetration (Zaitoun *et al.*, 1991), encapsulation to the penetrates radicle or necrosis (Zaitoun and Ter Borg, 1994). In these accessions we also found lower levels of germination than in the check. The other accessions studied in the petri dishes experiment showed levels of resistance to germination, but not to establishment.

The low level of broomrape attack of some lines can be explained by resistance to the shoot development. For instance, V-259 and V-37 allowed very little emergence of broomrape shoots in the field in the two more conductive years (96/97 and 97/98) but had higher numbers of non-emerged shoots. Also in pots all the tubercules found were in a very early stage of development (3). On the other side, other lines such as V-215, V-282- V-235 and V-35 displayed a high ratio of emerged/non-

emerged shoots in the pot experiment, indicating that those that are successfully established, do not find further restriction to it growth. Their ratio is even higher than in the susceptible checks. That can be explained by the lower competition for nutrients among the few established tubercules. However, in the lines for which we proposed resistance to development, the few tubercules established do not grow further even when they have little competition among them.

Four different mechanisms of resistance to *O. crenata* have been detected in the genus *Vicia*. The first one is a barrier to the germination of the *Orobanche* seeds. This is in contrast to data published in faba bean (Van Woerden et al., 1994) and in *V. atropurpurea* (Goldwasser et al., 1997) where the germination of *O. crenata* or *O. aegyptiaca* seeds, respectively, was higher in resistant than in susceptible lines. The second mechanism of resistance is a barrier to the establishment of the tubercule. The third is a barrier to the development of the broomrape shoot. One more mechanism, sporadically detected, is the presence of necrosis in the contact zone between the broomrape radicle and the host root. This necrosis have been previously detected in faba bean (Zaitoun and Ter Borg, 1994) and *V. atropurpurea* (Goldwasser et al., 1997). As several mechanisms of resistance seem to be operative in *Vicia*, it should be very interesting to combine some of them in the same lines and even more to transfer these mechanisms to the cultivated specie, *Vicia faba*.

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Table 1: Levels of broomrape attack in *Vicia* spp., in the field season 1995/96.

Species	N. of entries	Range[a] (%)	Species	N. of entries	Range[a] (%)
V. amphicarpa	1	0	*V. macrocarpa*	2	0-34
V. angustifolia	6	0-43	*V. megalotropis*	1	0
V. articulata	10	0-82	*V. melanops*	3	0-19
V. benghalensis	10	0-42	*V. michauxii*	3	0
V. bithynica	3	0-86	*V. monantha*	6	0-117
V. cordata	5	0-118	*V. narbonensis*	12	0-103
V. cracca var. cracca	2	0	*V. onbrychioides*	1	0
V. cracca ssp. stenophyll	1	160	*V. orobus*	1	0
V. cuspidata	1	0	*V. palaestina*	3	0-28
V. dalmatica	2	0	*V. pannonica*	12	0-104
V. disperma	2	0-19	*V. peregrina*	7	0
V. ervilia	9	0-57	*V. sativa*	33	0-214
V. fulgens	1	63	*V. segetalis*	1	16
V. galilaea	1	24	*V. sepium*	2	25
V. grandiflora	5	0-13	*V. serratifolia*	2	0-69
V. hirsuta	9	0-13	*V. sicula*	1	75
V. hybrida	4	0-75	*V. striata*	1	27
V. hyrcanica	2	0-24	*V. tennifolia*	3	0
V. incisaeformis	1	8	*V. tetrasperma*	2	0
V. johannis	5	20-103	*V. vicioides*	1	9
V. lathyroides	4	0-38	*V. villosa*	18	0-131
V. lutea	9	0-61			

[a] Maximum and minimum percentage of emerged broomrapes showed by accessions of each species, referred to the susceptible check, cv. Prothabon.

Table 2: Established broomrape in pot experiment[a].

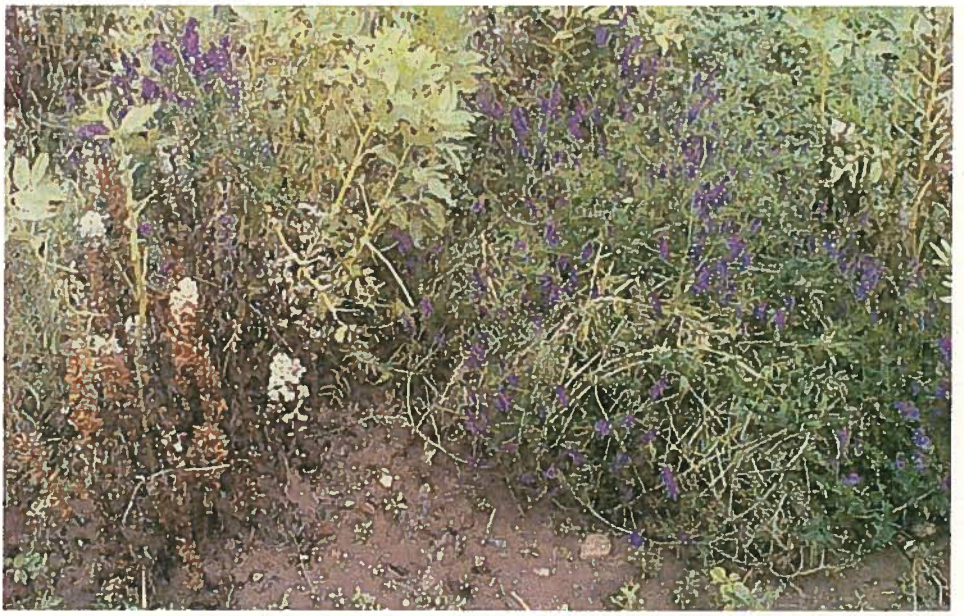
Accession	Species	Stage <4	Stage ≥4	Accession	Species	Stage <4	Stage ≥4
Messire	*Pisum sativum*	11.9	5.2	V-197	*V. dalmatica*	1.2	0.2
Prothabon	*Vicia faba*	13.9	1.4	V-233	*V. onbrychioides*	1.3	0.0
V-215	*V. hirsuta*	6.0	2.1	V-220	*V. michauxii*	0.9	0.3
V-282	*V. grandiflora*	5.5	1.4	V-37	*V. peregrina*	1.1	0.0
V-106	*V. peregrina*	3.8	0.8	V-97	*V. melanops*	1.1	0.0
V-235	*V. megalotropis*	2.8	1.5	V-128	*V. tetrasperma*	1.1	0.0
V-52	*V. hirsuta*	2.5	0.2	V-41	*V. sativa*	0.8	0.3
V-104	*V. peregrina*	2.1	0.5	V-155	*V. benghalensis*	1.0	0.0
V-194	*V. tennifolia*	2.5	0.0	V-163	*V. cordata*	0.9	0.1
V-35	*V. palaestina*	1.2	1.2	V-193	*V. tennifolia*	0.8	0.1
V-257	*V. michauxii*	2.0	0.3	V-284	*V. hybrida*	0.9	0.0
V-105	*V. peregrina*	1.8	0.1	V-259	*V. monantha*	0.9	0.0
V-190	*V. cracca*	1.7	0.2	V-214	*V. hirsuta*	0.6	0.3
V-44	*V. peregrina*	1.3	0.3	V-133	*V. villosa*	0.6	0.2
V-9	*V. lutea*	1.5	0.0	V-192	*V. tennifolia*	0.5	0.2
V-45	*V. peregrina*	1.3	0.1	V-109	*V. lutea*	0.5	0.0
V-196	*V. dalmatica*	1.3	0.1				

[a] Stages of development according to Ter Borg *et al.* (1994), 0 to 7 scale. Stage 4 indicates bud development.

Table 3: Establishment and germination of broomrape seeds in different entries of *Vicia* studied in petri dishes [a]

Accession	Species	N. of established broomrapes/ plant	% germination	% establishment [b]
Messire	*Pisum sativum*	26.4 a	53.2 a	33.7 ab
V-246	*V. serratifolia*	16.6 b	24.1 bc	38.3 a
V-41	*V. sativa*	14.6 bc	32.9 b	26.9 b
V-259	*V. monantha*	14.2 bc	19.3 c	40.5 a
V-37	*V. peregrina*	10.8 c	20.8 c	36.5 a
V-128	*V. tetrasperma*	4.8 d	9.3 d	36.9 a
V-52	*V. hirsuta*	4.2 d	21.7 c	18.2 c
V-133	*V. villosa*	2 de	19.2 c	7.7 d
V-193	*V. tennifolia*	0 e	4.3 de	0 d
V-196	*V. dalmatica*	0 e	0 e	-

[a] Data with the same letter per column are not significantly different (LSD, $p<0.05$).
[b] Percentage of those germinated seeds contacting the plant root that successfully established a tubercule.



RESISTANCE TO *Orobanche crenata* IN CHICKPEA

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Introduction.

Crenate broomrape (*Orobanche crenata*) is a major constraint for faba bean, field pea, lentils and various forage legumes that is widely distributed in the Mediterranean Region and West Asia. However, although chickpea is an important legume in the area and is known to be a host of *O. crenata*, it does not suffer important levels of infection (ICARDA, 1989; Bouhatous, 1987; Boulif, 1992). This has been ascribed to escape due to spring sowings, as it is known that a delayed sowing reduces the broomrape incidence, but we should be aware that with the continuous spread of the winter sowing practise, broomrape might appear as a problem (Fig. 1).

A preliminary screening has been performed to detect sources of resistance and to study the nature of the resistance.

Material and methods.

Ninety nine accessions, belonging to 11 species of *Cicer* were screened for broomrape resistance under field conditions during the seasons 1995-96 and 1996-97. Each line was represented by 1 meter row with 10 plants per row, surrounded by four rows of the *Vicia faba* susceptible check cv. Prothabon. Sowings took place early December. Final number of broomrape shoots per plant was counted at plant maturity. Also number of non-emerged broomrape tubercles per plant was assessed digging the plants. The number of emerged and non-emerged broomrape attachments per plant was referred to the mean of its four surrounding rows of Prothabon.

In addition, the reaction of 72 cultivars and breeding lines of *C. arietinum* was assessed the season 1997-98. In this occasion the susceptible checks were split over the plot, but no surrounding all the test lines. Only emerged shoots per plants were assessed.

Two selected accessions, plus the susceptible check were studied in pots and in petry dish experiments. Ten plants per accession were grown individually in pots filled with 1 litre vermiculite mixed with 25 mg (about 8000 seeds) of broomrape seeds. Three months after sowing the plants were extracted from the pots, the roots washed with water and the number of tubercles per plant counted. The petry dish method was similar to that described by Sauerborn *et al.* (1987). Five plants per entry were grown individually in petry dishes with 8 mg of conditioned *Orobanche crenata* seeds spread over a filter paper layer. 35 days later final number of tubercles per plant was counted. Also about 500 seeds were studied and grouped according to their stage of development (non-germinated, germinated and germinated establishing a tubercle) in order to calculate the percentage of germination and of establishment. Viability of the *Orobanche* seeds was studied previously with the TTC method (López-Granados and García-Torres, 1996).

Results and discussions.

Resistance was common in wild *Cicer* accessions but also in entries of *C. arietinum* (table 1). All the accessions studied of *C. bijugum, C. canariense, C. echinospermum, C. judaicum, C. macracanthum, C. multijugum, C. oxyodon, C. pinnatifidum* and *C. songoricum* were highly resistant. Most of the accessions of *C. reticulatum* were also highly resistant, although some had small levels of infection, but considerably reduced with respect the susceptible check. The total number of broomrape tubercles was relatively high in some accessions, but most of those shoots did not develop further and did not emerge. This was particularly evident in accession ILWC81. This could be explained by the production of secondary metabolites by the plant that arrests the development of the broomrape shoots. In this line, Wegmann *et al.* (1991) suggested that an increased production of phytoalexins (maackiain and medicarpin) in the resistant chickpea cultivar ILC280, compared to the susceptible FLIP81/35W. But we could also explain that by a late establishment that does not allow the broomrape to emerge. We assessed the number of broomrapes at plant maturity to allow the emergence of broomrapes from late genotypes, but still in many occasions they did not emerge. This could be expected in genotypes with delayed emergence and root development, but short life cycle, so they can mature quickly, and the broomrape does not have time to emerge. A source sink competition between the parasite and the pods has been suggested by Manschadi *et al.* (1997) in faba beans. Thus an early flowering genotype would have an advantage over broomrape due to earlier pod-setting and maturity, which would restrict the dry-matter partitioning into parasites. In a similar way, a late establishment would have disadvantages to the parasite in terms of competition. More detailed studies on the cycle

of the plant and its implications on the establishment and development of *Orobanche*, are needed to clarify these aspects.

C. reticulatum is cross-compatible with cultivated chickpea (Ladizinsky and Adler, 1976). It is also possible to obtain successful crosses with *C. echinospermum*, even though their cross progenies have reduced fertility (Pundir and Megesha, 1995). Thus, the useful levels of resistance found in both species are accessible for chickpea breeding by conventional methods. The transfer of the resistance of the remaining species would require development of new in vitro regeneration and genetic transformation methods.

Only one accession of *C. yamashitae* (ILWC53) was very susceptible. It is remarkable that also in other screenings for resistance to *Fusarium* wilt and cold *C. yamashitae* has been considered the least important species in terms of resistance available (Singh and Saxena, 1997). Accessions ILWC108, ILWC113, ILWC115, ICCW6 and PI499777 of *C. reticulatum* and CA2138, CA2225, 561 and P2245 of *C. arietinum* were moderately susceptible. The fact that we only name the susceptible accessions remarks the extension of the resistance available in the genus.

The pot (table 2) and petry dish (table 3) experiments probed the resistance of the 2 accessions studied. The final number of attachments were extremely reduced compared to the susceptible checks. Pot and petry dish screenings are faster and cheaper than the field one, prevent the escapes due to an uneven distribution of the *Orobanche* seeds in the soil and reduce the environmental influence. Also the effect of plant vigour and root length can be easier determined. A further advantage would be the feasibility of testing with different *Orobanche* populations and species. Linke *et al.* (1991) developed a similar pot method to screen for resistance to broomrape in chickpea. They reported reductions of up to 100 % of seed yield in pot experiments. The number of emerged shoots in the checks in our pot experiment was lower than we would expect from field experiments. That might be due to the substrate used, vermiculite, that was satisfactory at the early stage of the experiment, but the plants grew poorly after two months. Thus the use of a different substrate, like that used by Linke *et al.* (1991) would be recommended for future long term pot studies.

From the petry dish experiment it appears that this resistance is mainly due to low induction of germination of the *Orobanche* seeds. Thus, biochemical studies on the amount and nature of the germination stimulants excreted by roots of these *Cicer* accessions are needed. It could also be possible that this resistance were not only due to lack of excretion of stimulants, but to production of germination inhibitors. Also resistance to establishment is suggested in accession C-336 of *C. oxyodon*, but

not in accession C337 of *C. pinnatifidum*. However the differences between C-336 and C-337 were not significant. The number of germinated seeds was so low that it was difficult to study the establishment in a representative sample. This is reinforced by the results of the pot experiments where no tubercles were formed on C-336.

Linke *et al.* (1991) found in pot experiments with *Cicer arietinum* more underground attachments than emerged shoots. At higher parasitic seed densities, the total number of attachments increased, but also the proportion of underground attachments, indicating a competition among underground attachments at higher seeding densities. A small amount of attachments were established on C-337. But in this highly resistant accession the ratio emerged/non-emerged was higher than in the susceptible accessions, indicating that the few established tubercles had less competition and could emerge easier. That suggests that resistance acts in an early stage of the infection, not being based on restriction of tubercle development due to production of secondary metabolites. The reduced success on establishment of the reduced number of germinated seeds results in a lower number of attachments. Thus, the competition among the few established tubercles is less and they can grow further and emerge.

At southern Spain we are used to see low levels of broomrape infections in winter sowings, that was never considered to be a problem in terms of yield. However, the season 1997-98 was very conducive for the disease and we had unusually high and consistent infection on experimental plots of the cv. Athenas, compared to the low levels of infection on cv. Fardon (Fig. 2). Thus, we learned that broomrape can be a problem on some years and cultivars, but also that resistance is already available in commercial cultivars. We conclude that broomrape could be a severe problem on winter chickpea on some particularly conducive years. Resistance is common both in breeding lines of *C. arietinum* and in its wild *Cicer* relatives. The situation is far easier than on faba bean, peas and lentils where resistance is extremely scarce, and a huge effort is proving to be needed for the obtention of a variety with a satisfactory levels of resistance (Cubero, 1991).

There is a report of *O. foetida* infection on chickpea in Tunisia (Kharrat *et al.*, 1992), thus attention should also be paid to this species. It has been reported that the resistance of faba bean to *O. crenata* is not effective to this aggressive population of *O. foetida* found in Tunisia (Anonymous, 1991), so it will be desirable to perform resistance screenings also to this population of *O. foetida*, that although at present seems to be restricted to a particular area in North West of Tunisia, could easily spread. There are natural populations of *O. foetida* spread over South-West Europe and North Africa (Pujadas, 1999), so there is the risk that they may become also a problem. Virulence of those natural populations should be compared with the Tunisian one.

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Table 1. Field reaction to crenate broomrape *(Orobanche crenata)* of a collection of *Cicer* spp.

Species	number of accessions studied	Emerged shoots per plant range (%)[a]	Non-emerged Tubercles per plant range (%)[a]
C. arietinum	72	0-46[b]	n.d.
C. bijugum	16	0-17	0-26
C. canariense	1	0	0
C. echinospermum	19	0-5	0-47
C. judaicum	8	0	0-18
C. macracanthum	1	0	0
C. multijugum	1	0	0
C. oxyodon	2	0	0
C. pinnatifidum	15	0-12	0-59
C. reticulatum	31	0-27	0-130
C. songoricum	1	0	0
C. yamashitae	4	0-75	0-43

Footnotes:

[a] Range of values found in the species referred to the mean of the four surrounding rows of the susceptible faba bean check cv. Prothabon (=100%). Seasons 1995-96 and 1996-96.

[b] Referred to the mean of the susceptible check split over in the plot (=100%). Season 1997-98.

Table 2. Total number of broomrape attachments per plant in ot experiments

Accession	Species	non-emerged	Emerged	Total
Messire	*Pisum sativum*	9.2 a	0.18 b	9.36 a1
Prothabon	*Vicia faba*	6.91 a	0.67 a	7.58 a
C-336	*C. oxyodon*	0 b	0 b	0 b
C-337	*C. pinnatifidum*	0.18 b	0.09 b	0.27 b

Footnotes:
[1] Letters in common per column indicate that differences are not statistically significant (P=0.05)

Table 3. Components of resistance to *Orobanche crenata* in petry dishes experiments

Accession	Species	number of tubercles/plant	% germination	% establishment[1]
Prothabon	*Vicia faba*	13.3 a2	37.6 a	21.1 a
C-336	*C. oxyodon*	0.5 b	1.9 b	4.5 b
C-337	*C. pinnatifidum*	0.2 b	0.1 b	20 ab

Footnotes:
[1] % of those germinated broomrape seeds that formed a tubercle on the plant root.
[2] Letters in common per column indicate that differences are not statistically significant (P=0.05)

BREEDING FOR *Orobanche* RESISTANCE IN FABA BEAN & LENTIL

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Introduction.

In the Mediterranean region two species: *Orobanche crenata* Forsk. and *Orobanche aegyptiaca* Pers. cause a considerable yield losses in faba bean (*Vicia faba* L.) and lentil (*Lens culinaris* Med.).

On faba bean estimates of actual infested area in Egypt given as 65% of the cultivated area of Behera Governorate of the North Delta with yield losses of about 19,000 tons (Zaitoun 1990). The infested area extended to cover 18-86% with yield losses of 7-80% (Korachi 1996). In some governorates (Minofia) of the Nile Delta, 1-4 *Orobanche* spikes/faba bean plant decreased seed yield by 15-53% (ARC, 1998). In Morocco the infested area was estimated as 113,000 ha (Sauerborn 1991) and yield losses from 12 to 60% (Zemrag 1993). The most recent estimate indicated that 32.6% of the area is infested and yield losses equal to 8.6 million US$ (Geipert *et al.*, 1996). In Spain about 50,000 ha, in Syria (Parker 1994) and in Portugal 13,500 ha are being attacked by *Orobanche* (Sauerborn 1991).

In Tunisia *Orobanche foetida* Poir. distribution has expanded and is severely attacking faba bean (Kharrat et al 1992).

On lentil two species of broomrape, *Orobanche crenata* Forsk and *O. aegyptiaca* Pers., are important parasites, particularly in Morocco, Syria and Turkey. In Syria both species are important. In a survey of 115 lentil fields, 55% of fields were infected with the range of 2-80% infected plants (Bellar and Kababeh 1993). In Turkey *O. aegyptiaca* predominates and a major infestation was recorded in the main growing provinces of Shanliurfa and Mardin of Southeastern Anatolia, with the level of infestation so high in some fields that they will soon be out of production without appropriate control measures (Bayaa *et al.* 1998). In Morocco only *O. crenata* is found on lentils. Yield losses was estimated as 10% in Syria and 10% in Morocco (Sauerborn 1991).

Control Measures in Faba Bean and Lentil.

Hand weeding: Can only be recommended for very limited infestation to prevent any further increase in the parasite population (Garcia - Torres 1994), or reduction in seed bank (Linke 1992). This control method is generally used where no other feasible means of control and cheap labour, such as in India, where the hand tool 'spear' is used by farmers of tobacco (Krishna Murty and Nagarajan, 1991; Krishna Murty and Raju, 1994). In Tunisia no control measures are used by farmers, other than avoiding bean planting. In Syria, control is mainly by delayed planting (70%) and by hand pulling (10%).

Chemical control: Results over three years, 1980-1982, under ICARDA/IFAD Nile Valley Projects (1983) indicated that in Egypt glyphosate applications (64g.a.i./ha) as a post-emergence foliar spray at the beginning of flowering (three sprays, three weeks intervals resulted in reduction in parasitism within a range from 97% to 100% and increased seed yield from 34 to 124%. Glyphosate application, was recommended for large-scale in faba bean, under heavy infestation with *Orobanche* in Spain however, the new herbicide, imazethapyr, has now been approved and is about to come into use in Spain (Parker 1994). In Morocco glyphosate is used for control but is not readily adopted by farmers. The herbicide imazethapyr 150 g./ha applied as pre-emergence gave the highest seed yield (2.06Tm/ha) compared to (0.41 Tm/ha) in the control (Saber *et al.* 1994). In lentil an attempt has been initiated in ICARDA to screen some herbicides on lentil, results from a single season (1997/98) indicated that the new herbicide Imazapic (Cadre) is very promising as it reduced the infestation level by 80%.

Late sowing: In Egypt late sowing during the last week of November and in the first week of December gave the highest faba bean seed yield under heavy infestation with *Orobanche* (ICARDA/IFAD – Nile Valley Project – 1983). The same findings were also reported by Al-Menoufi (1984) and in Spain (García-Torres 1994). Plant lentil delayed sowing was also useful component part of an integrated control package as mentioned by Linke and Saxena (1991).

In Syria late sowing has proved effective with narbon vetch and field pea as well as faba bean and lentil (Linke 1992). The importance of low temperature on germination and early development of *Orobanche* was markedly effective in decreasing the *Orobanche* dry weight with delayed sowing date. There was a decrease in crop biomass by delaying the sowing date, but the reduction of the parasite was stronger and over all results were positive.

Cultural practices: The use of crop rotation, intercropping faba bean with some other trap crops, such as flax, and fenugreek significantly reduced the infestation level with *Orobanche* (Al-Menoufi, 1994). The infestation level was reduced when crop rotation included rice, due to water flooding (Sauerborn and Saxena 1986).

Solarization: Results of Abu-Irmaileh and Thahabi (1997) in Jordan indicated that seed germination of *Orobanche cernua, O. crenata* and *O. ramosa* was sensitive to solarization. Germination of *O. cernua* was the most affected. Solarization with clear polyethylene (PE) sheets for two months was more effective. Abdalla and Dabrowski (1997) in Sudan reported that soil solarization has eliminated *Orobanche* and majority of weeds from the treated plots. The tomato crop yield has increased from 7.8 on untreated control plots to 21.1 Tm/ha on plots treated with solarization in 1995/96.

Breeding for *Orobanche* Resistance.

Germplasm evaluation and selection.

An early study of Elia (1964) indicated that variability to *Orobanche* infestation was found among 15 Italian landraces. He reported that one cultivar 'Favino palombino' was comparatively resistant, and noticed that resistance was associated with dark-coloured seed coat. Kasasian (1973) in UK stated that large seeded faba bean cultivar 'Express' was the most resistant among a collection of 53 cultivars. Results of Boorsma (Unpublished data) in Morocco indicated that the black seeded F331 was partially resistant. Basler and Haddad (1978) in Syria recorded 36 faba bean lines resistant to *Orobanche*. In Egypt 22 families derived through a 3-year cycle of individual plant selection in an F7 line of the cross: Rebaya 40 (commercial variety) X F216 (land race) along with seven commercial varieties and land races were evaluated for *Orobanche* resistance/tolerance (Nassib et al. 1978). Results indicated that Family 402 expressed a higher level of tolerance to *Orobanche* than the other selected families or check cultivars. Results of further studies pointed out that on average it had 57.5% less *Orobanche* spikes/hill and nearly four times more *Orobanche*-free plants than the commercial variety Giza 2. Results (Nassib et al. 1982) also showed that the seed yield and harvest index of F.402 were 5.8 and 2.2 times those of the check cultivar Giza 4, respectively under heavy *Orobanche* infestation.

A collection of 209 Egyptian land races was studied by Abdalla (1982). Results revealed wide variability between and within collected material. Results of Fischbeck

et *al.* (1986) indicated that reaction of faba bean entries to *Orobanche* infestations may due to genetic differences among populations of *Orobanche crenata*. Radwan et *al.* (1988a) stated that selection between and within land races proved to be more effective than between genetic stocks. A wide variability to *Orobanche* infestation was observed between faba bean populations derived from different origins (Perrino et *al.* 1988) and environmental conditions affected tolerance to *Orobanche* infestation. Radwan et *al.* (1988b) in Egypt found that parasitic accessions were quite different in their influence on host genotype and host-parasite relationship and were largely dependent on environmental conditions. Rehberg and Alkamper (1990) recorded that 'Alfred' cultivar was very susceptible and Giza 402 was the most resistant. The level of parasite infestation within faba bean varieties ranged from 0.0>100, thus they emphasized the need for single plant selection for breeding against *Orobanche*. Results of Zaitoun (1990) indicated that Giza 402 was tolerant to *O. crenata*, however, reaction of Giza 3, Roumy, Reina blanca and Sevila Giant to Orobanche infestation, ranged from susceptible to highly susceptible. Results of Attia (1992) in Egypt showed that the lowest *Orobanche* infestation on the new breeding line 674/155/85 and the highest occurred on Giza 2. Giza 402 did not differ significantly from 674/155/85. The breeding line 402/29/84-1 out yielded all genotypes followed by Giza 402.

In lentil a total of 1774 germplasm accessions were screened under field conditions against *Orobanche crenata* in northern Syria from 1979 through 1981. Reaction was recorded during late pod filling (Erskine and Witcombe, 1984). Field screening revealed repeatable differences in the number of broomrape inflorescence per plot.

Screening methods.

In Faba bean: Screening methods for *Orobanche* resistance are being conducted under heavy natural or artificial infestation. A rapid, simple and applicable screening method is needed by breeders.

In Lentil: Field screening is done in highly infested soil. The maximum score in any replication was used for analysis. A comparison between the data of different years was made by adjusting annually in relation to the overall population mean. The scale employed was 1 = more than one standard deviation below the population, mean; 2 = within one standard deviation below the population mean; 3 = within one standard deviation above the population mean; 4 = more than one standard deviation above the population mean.

A rapid laboratory test for screening lentil genotypes for resistance to *Orobanche* sp. was developed with lentils growing in attapulgus clay-filled petri dishes between glass microfibre filter paper sprinkled with broomrape seed (Sauerborn *et al.*, 1987). The number of parasitic attachment is counted after 35 days of incubation at 25/20 °C (day/night).

Using this method, the reaction of the lentil accessions most resistant in the field was re-examined under laboratory conditions. No significant differences among accessions were found in the number of infections of *O. crenata* per unit length of root. The low incidence of *Orobanche* infection on the roots of 'resistant' accessions in the field was probably due to poor root growth. Despite further extensive screening in petri dishes, resistance to *Orobanche* has not been found in the cultigen. Screening has continued with the wild *Lens* spp. but resistance has not been found.

Development of New *Orobanche* Resistant Cultivars.

In Egypt.

Considerable attention was given to exploit the genetic resistance of F402. Khalil *et al.* (1994) discussed the results of breeding studies conducted under the Nile Valley Project (NVP) in Egypt during the period 1986-1993. Results indicated that four breeding lines: 402129/84, 674/154/85, 674/155/85 and X-843 were significantly less infested by *Orobanche* and gave higher yields than the susceptible check cultivars. The breeding line 402/29/84, which was selected from the commercial cultivar Giza 402 yielded 60% more than the original population (Giza 402). However the other two lines 674/154 and 674/155/85, both selected from a cross between Giza 402 and BPL 561 (from ICARDA), produced similar yield to the resistant check (Giza 402).

The breeding line X-843 was selected through multi-crosses including as parents: Giza 402, ILB 938 (resistant for chocolate spot), S. Giant (Spanish land race), NA29 (from Holland) and Egyptian land races. This breeding line produced significantly higher seed yield than 402/29/84 and both were similarly resistant to *Orobanche*.

These findings indicate the value of:

1. Individual plant selection in the original population (such as Giza 402).
2. Combination of genes from different genetic resources through targeted multicrossing and selection.
3. Exploitation of genes for resistance to other stresses (different mechanisms) to build up and raise the level of resistance to *Orobanche*.
4. Screening and evaluation under heavy natural infestation is highly effective in identify lines with resistance.

Results of yield trials and on-farm yield trials on the farmers' fields were discussed by Khalil *et al.* (1995), and recorded in NVRSRP (1998), indicating that families selected from cross No. 674 and those selected from the commercial cultivar Giza 402 yielded 82.6% seed yield more than the check cultivar Giza 3 and both are now released to the farmers under the commercial names: Giza 674 and Giza 429, in Middle and Upper Egypt. The breeding line: X-843 has recently released for commercial use under: Giza 843 in the new Delta of Egypt for resistance to *Orobanche* and to chocolate spot (*Botrytis fabae*), and early maturity.

In Spain.

Statistical analysis of several segregating generations showed the quantitative genetic control and some degree of resistance (Cubero, 1982). Hernández (1987); and Cubero and Hernández (1991) stated that the additive genetic system is important in resistance to broomrape; susceptibility is dominant over resistance and in one case genetic complementation was found. A polygenic system was also mentioned by Khalil et *al.* (1994) and Attia (1998).

In Spain one genotype VF1071 was selected from F402, and used in breeding to develop a well adapted, high yielding cultivar 'Baraca', to registered for high yield and *Orobanche* resistance (Cubero et al., 1992). Zaitoun and Ter Borg (1994) and Van Woerden (unpublished data) reported that its level of resistance is higher than Giza 402 (original cultivar). A similar finding was reported in Egypt where Giza 429 (new released cultivar) was selected from Giza 402 (origin) by individual plant selection, and recorded a higher level of resistance than the original population (Khalil *et al.* 1994).

Results of Attia (1998) indicated that Giza 402, Giza 429, X-843, Line 101, Cairo 241, BPL 536 and Pop. 11 were tolerant to *Orobanche*. Results of diallel analysis indicated that variance of General Combining Ability (GCA) was greater than that of Specific Combining Ability (SCA) for plant height, dry weight, except *Orobanche* dry weight, indicating that the additive variance was predominant for conditioning host plant trait under heavy infestation with *Orobanche*.

Stability of *Orobanche* Resistance.

Results of stability analysis indicated that in Andalucia (southern Spain) the most stable genotypes for resistance were not the same as those for yield (Flores *et al.*, 1996). Three lines, L1, L2 and VF1071, were by far the most *Orobanche* resistant materials, but their yields showed low stability among environments. The authors stated the need for improvement in the yield stability of the three resistant genotypes.

Results of Attia (1998) indicated that significantly unestable genotypes for seed yield were the *Orobanche* tolerant genotypes: X-843, BPL 536, Cairo 1 and Giza 674.

Integrated Control of *Orobanche*.

In faba bean.

Under the Nile Valley and Red Sea Regional Program (NVRSRP) in Egypt (1998) an Integrated Package for *Orobanche* control was developed and successfully applied on the farmers fields, the package recommend the use of:

1. Clean seed of the resistant or tolerant cultivars:
   Giza 402, Giza 429, Giza 674 (Middle and Upper Egypt) and Giza 843 (Nile Delta).
2. Reduced recommended dose of glyphosate (34 g.a.i./ha) + NPK (1:1:2), three times (2-3 weeks intervals), at the on set of flowering stage.
3. Late sowing around November 15th.
4. Planting on non-tilled soil after cotton and maize or rice, in cotton and rice rotation system.
5. Applying *Rhizobium* inoculation of the seed, fertilizers, insect control, foliar disease control, irrigation, weeding... etc. as recommended.

Results of the last five years (1994-1998) indicated that over all Egypt the *Orobanche* control package increased faba bean yields of infested demonstration plots by 1.47 t/ha (88.3%) associated with reduction of number and dry weight of *Orobanche* by 32.8 spikes/m^2 (84%) and 57.5 g /m^2 (82.1%) respectively.

In lentil:

Many methods for *Orobanche* control have bean tried; individually no method has given 100% control of the parasite under high infestation levels. The best combination of control methods has been used. Such components included:

1. Delay sowing.
2. Use of herbicide.
3. Early flowering cultivar adapted to late sowing.

Thus in the absence of usable resistance to broomrape in the crop, a significant sowing date x genotype interaction (Silim *et al.*, 1991) is being exploited to avoid *Orobanche* problems.

Recently, exciting but preliminary results on control of *Orobanche* were obtained with the application of 'imazapic', which reduced infestation by 80% on average in early sown local cultivar (Bayaa and Erskine unpublished date).

Future Plan of Work.

In faba bean:

1. Identification of new genetic resources for *Orobanche* resistance.
2. Exploitation the available resistant genetic resources for raising the resistance level through: multicross breeding, back crossing and recurrent selection.
3. Development of improved faba bean populations to be used by NARs, under different ecological environments.
4. Breeding for resistance to herbicides.
5. Biological control by using insects (*Phytomyza* sp.).
6. Development of a rapid screening technique in faba bean.

In lentil:

1. Does the availability of a selective herbicide for use at low rates reduce the priority for breeding for resistance to broomrape in lentil?
2. Should the availability of a transgenic system for lentil be used to incorporate resistance to a selective herbicide to allow broomrape control?
3. Use of herbicides as seed treatment.

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Orobanche RESEARCH ACTIVITIES ON FABA BEAN IN TUNISIA

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Broomrape (*Orobanche* sp.) is one of the most important parasites on faba bean (*Vicia faba* L.) in Tunisia. Two angiosperm species *Orobanche crenata* Forsk. and *O. foetida* Poir., are commonly encountered in Tunisian faba bean fields (Kharrat *et al.*, 1992). The damages of *O. foetida* on faba bean has been reported only in Tunisia. Populations of *O. foetida* are more agressive on faba bean than on other food legume crops (Kharrat *et al.*, 1994). Among the major food legumes, only pea (*Pisum sativum* L.) escapes to *O. foetida* infestation, however it is heavily infested by *O. crenata.*

The geographical distributions of *O. crenata* and *O. foetida* are different. *O. crenata* is observed mainly in the northern and central-eastern regions, whereas *O. foetida* is noticed in the central North of Tunisia (Figure 1).

Research efforts on the control of broomrape on food legumes, in the Mediterranean area, was concentrated mainly on *O. crenata / V. faba* system. Great progress has been achieved in developing resistant varieties mainly in Egypt and Spain (Nassib *et al.*, 1982; Cubero *et al.*, 1992; Khalil *et al.*, 1994).

Yield losses caused by broomrape on faba bean could reach more than 80% in heavily infested fields. The Tunisian food legume national research programme has given a great attention to this problem and started a research activity aimed to:

- Identify infested areas with broomrape
- Test different control measures (chemical, agronomic, ...)
- Identify sources of resistance to *O. feotida*
- Evaluate the reaction to *O. feotida* of international elite lines that have been previously selected for their resistance or tolerance to *O. crenata.*
- Select faba bean lines resistant or tolerant to *O. foeitda* and adapted to local conditions.
- Develop integrated control package.

Achievement of the programme.

Chemical control.

Different herbicides have been tested to control *O. foetida* on faba bean. The best results are obtained by the applications of glyphosate at budding stage and 15 days later. Imazethapyr has given satisfying results when applied at pre-emergence (Kharrat and Halila, 1994, 1996). Seed treatment with imazethapyr reduced partly orobanche shoots on susceptible cultivars but the results seem to be affected by climatic conditions.

Agronomic practice.

Delaying sowing to mid-December increased yield compared with normal sowing date (mid-November). The increase of yield was due to lower infestation with broomrape and reduced dry weight of the parasite (Kharrat and Halila, 1994).

The inter-cropping faba bean crop with fenugreek (*Trigonella phoenum graecum* L.) gave contrasted results. Fenugreek reduced infestation of broomrape on faba bean only in wet conditions (Kharrat and Halila, 1996). In dry season competition between faba bean plants and fenugreek does not allow to improve the faba bean yield.

Breeding program.

Breeding for resistance to *Orobanche* is tedious. We have installed clinic nurseries where introduced material selected for its resistance to *O. crenata* is monitored for its reaction to *O. feotida.* Introduced lines are currently available from Egypt, Spain, ICARDA and Morocco. These lines showed some resistance to *O. feotida* but the level of this resistance is better for *O. crenata* (Kharrat *et al.*, 1994). Moreover, they appeared very susceptible to foliar diseases (i.e. Botrytis and Ascochyta blight).

The best resistant lines are used as genitor for introducing resistance genes in cultivars adapted to Tunisian conditions.

In collaboration with ICARDA, the food legume research programme of INRAT (Tunisian National Research Agricultural Institute) started to evaluate the world collection of faba bean pure lines (BPL) maintained in this centre. Out of this evaluation some lines resistant or tolerant to *O. foetida,* have been identified.

The faba bean programme has developed resistant faba bean small seeded lines with better level of resistance to *O. foetida* and foliar diseases and more adapted to local conditions. These lines had higher yield than the check (local populations) in fields infested with *O. foetida* (table 1) Some of these lines are now in increase for release.

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Table 1: Number and dry weight of Orobanche shoots per plant and seed yield of faba bean lines tested in infested field with O. foetida at Beja in 1997/98.

Lines	NO/P	DWO/P	Seed Yield (kg/ha)
XBJ90.03-16-1-1-1	1,0	8,6	2474
XBJ90.04-2-3-1-1	1,7	3,1	1815
Local Small	9,0	11,8	133
Local Large	11,6	19,3	459
Aguadulce	11,6	21,1	482

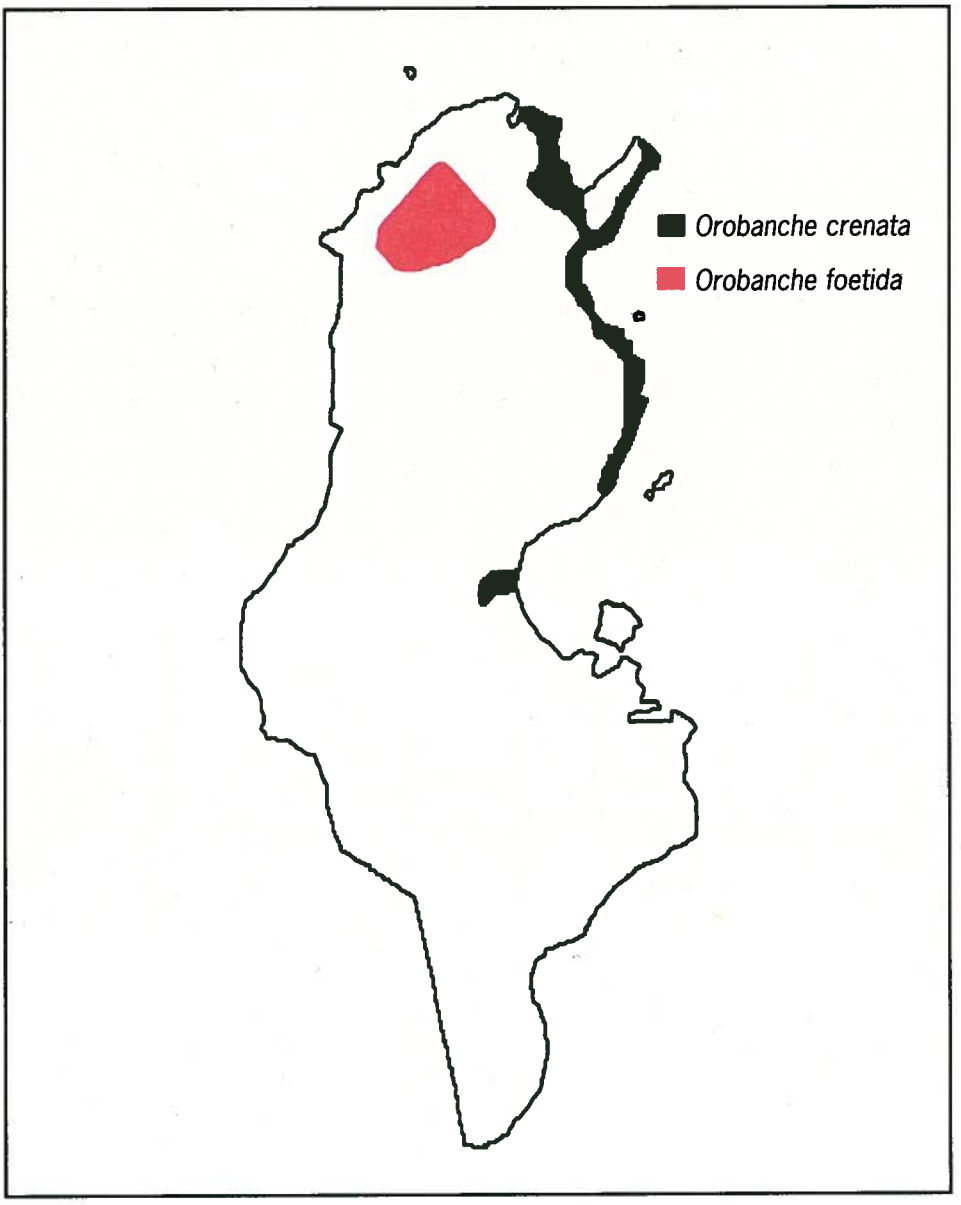
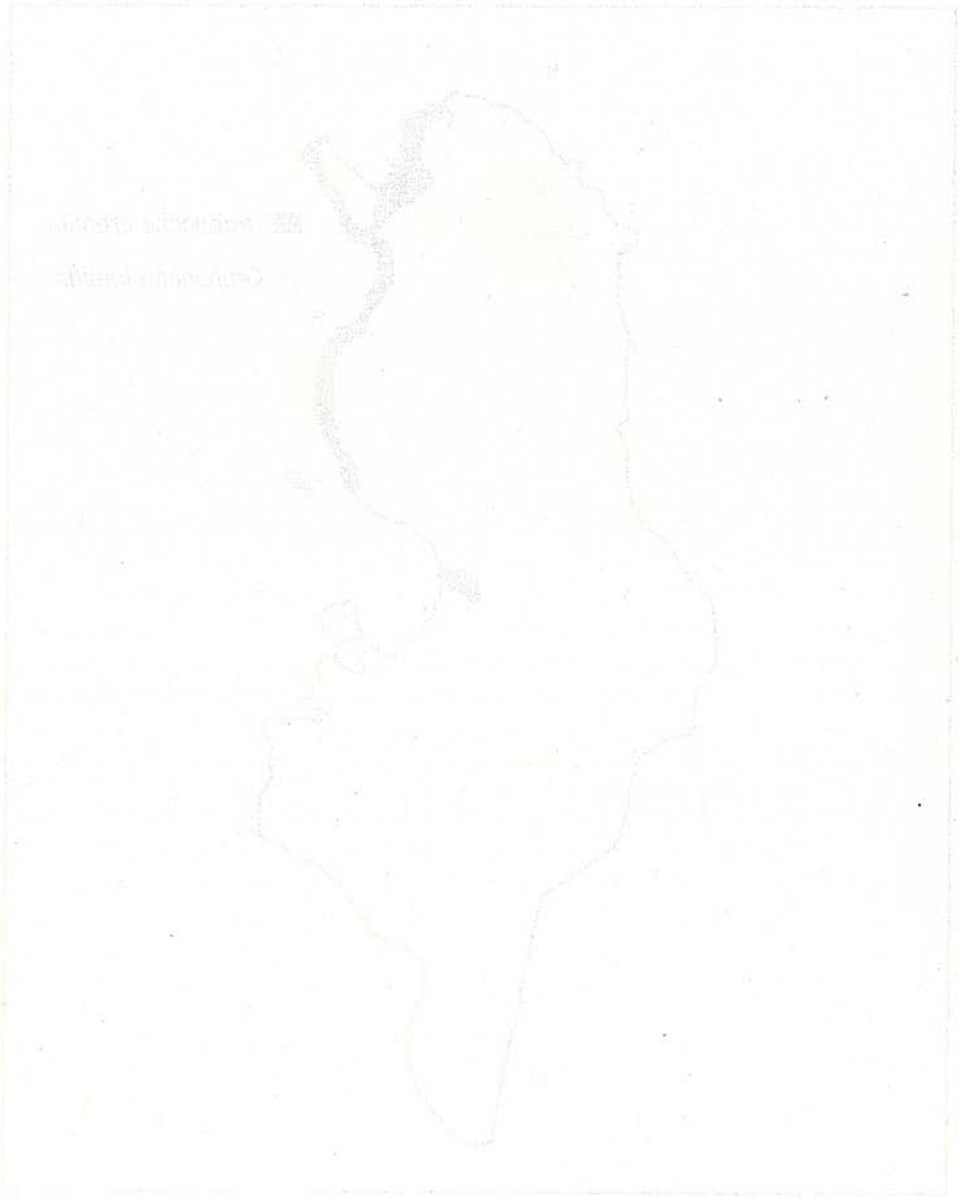


Figure 1: Infested areas with *O. crenata* and *O. foetida* in Tunisia.



BROOMRAPE (*Orobanche crenata*) AS A MAJOR CONSTRAINT FOR PEA CULTIVATION IN SOUTHERN SPAIN

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Introduction.

Dry pea (*Pisum sativum* L.) is a protein source crop of major economic importance at world level. Acreage in southern Spain has been traditionally low, although pea crop has a high potential in winter sowings. However, even after Governmental efforts to promote pea cultivation, the fact is that pea acreage is very limited. It increased significantly from 470 Ha in 1991-92 to 32.000 Ha in1995-96, but fell to less than 8.000 Ha since then. The 1991-96 period was characterised by an unusually severe drought (<300 ml during the crop season, versus >600 ml in normal years) that were somehow tolerated by pea giving an acceptable yield. Pea was considered a good alternative to the traditional wheat - sunflower rotation in dryland conditions. The drought was over in the season 1995-96, but the yield were much lower due to sanitation problems. This season was characterised by a rainy winter and a rather cold and humid spring, thus being very conducive to Ascochyta complex, but also to a novel severe pea problem, the occurrence of crenate broomrape (*Orobanche crenata*). Farmers had little experience with the pea crop and used to consider it a low input crop, paying little attention to quality and sanitation of the seed used, and to crop management. They were not aware of the infestation and had little knowledge of the potential crop losses. No breeding effort had been done for these conditions, so the cultivars were very susceptible. Thus, consequences for yield were dramatic. About 80 % of the fields were not harvested. But, what might even be worse, the *Orobanche* soil seed-bank increased greatly.

Pea is known to be a host of *O. crenata*, but broomrape has not been considered a potential problem in any review, and was not even mentioned as a curiosity (Hagedorn, 1984; Ali *et al.*, 1994; Cousin, 1997). *Orobanche* infectations can be

devastating to crops and remove otherwise productive land from effective use for very long periods of time. *O. crenata* infestations of pea have not been a major problem in European agriculture, but this parasite has begun to take hold in southern Spain (García-Torres *et al.*, 1996; Rubiales *et al.*, 1998), Morocco (Mabsoute and Saadaoui, 1996), Egypt (Korashi *et al.*, 1996) and Israel (Bernhard *et al.*, 1998). It is potentially the major constraint for pea cultivation in the Mediterranean area and Middle East, where the problem of broomrape is dramatic due to its broad distribution in the area, the long survival of the seed-bank in the soil and the extreme susceptibility of the cultivars available to the farmer. Yield loss can be huge, as high as 80 % (Korashi *et al.*, 1996) or even 100 % (Bernhard *et al.*, 1998). *O. crenata* is also a major constraint for faba bean, vetches and lentil. It has been reported also on chickpea, lettuce, safflower, geranium, camomile and carrot.

Material and methods.

Incidence of major diseases was monitored on pea fields during seasons 1995-96 and 1996-97. In addition, 610 accessions of *P. sativum* ssp. *sativum* and 110 of other subspecies were screened for broomrape resistance under field conditions at Córdoba in a heavily infested field in the season 1996-97. The final number of emerged broomrape shoots per plant was determined at crop maturity and expressed as a percentage of the mean of its four surrounding rows of the susceptible check cv. Messire (=100%). Resistance of selected lines was studied under field conditions in a second year (season 1997-98). Some of them were also studied in pot experiments in a greenhouse.

Results and discussion.

Ascochyta blight (*Mycosphaerella pinodes*) infections were high and thus, should be regarded of importance, after broomrape. Powdery mildew attack could be high on some genotypes, but tended to appear rather late. It could be of importance on some years. Little bacterial blight symptoms were detected on some lines early in the season, but did not progress none of the years. Downy mildew symptoms could be detected late in the season, but did not extend. Broomrape appeared as the major biotic limiting factor for pea production in the area (Fig. 1).

The reaction to broomrape of the test lines in the field in the season 1996-97 ranged from very susceptible to very resistant (number of emerged broomrape sho-

ots per plant ranging from 440 % to 0 % of that of the susceptible check cv. Messire). Most (about 90 %) of the accessions studied were very susceptible. Forty five *P. sativum* ssp. *sativum* and twelve wild *Pisum* accessions showed low levels of attack in the field (less than 10 % of Messire). The season 1997-98 was rather unusual in southern Spain, characterised by soft and rainy winter and fresh and rainy spring. Such years are known to be more conductive to the disease. Thus, the establishment of broomrape was higher, but whereas some of the lines that were selected in the season 1996-97, remained with very low levels of infection in 97-98, others were severely infected. In some of them, although the final number of attachments was high, they emerged late. This could be ascribed to weak levels of resistance in these lines, that would suffice to arrest bromrape attachment and/or development in normal years, but would not be enough in very conductive years and heavily infested soils.

Pot experiments confirmed the resistance of some of the accessions and showed that different mechanisms of resistance might be operating, preventing infection at different stages, from establishment till bud elongation. It is still necessary to study the stability of the resistance described here against different *Orobanche* populations.

There have been previous reports on incidence of *O. crenata* on pea in southern Spain, but it was not considered a major problem. de Oliveira-Velloso (1990) considered pea to be little susceptible compared to lentil, broad beans and vetches. He observed low incidence on pea during 1987, 88 and 89. However, Arjona-Berral *et al.*, (1987) found high infestation in artificially inoculated plots, indicating the susceptibility of pea, in agreement with our observations. This suggests that the fact that the broomrape problem did not arise in commercial fields before was just a lucky event, but the problem was to appear as soon as the pea acreage increased, as, first, most of the cultivars available are extremely susceptible; second, the weather conditions are usually conducive to the disease; third, crenate broomrape is very common in Mediterranean region, and maintained both by the tradition of legume cultivation and the fact that it has a broad host range; fourth, broomrape seeds can be dispersed short distances by wind, but also long distances mixed in pea seed lots; fifth, soil seed-bank can increase very rapidly, as a single broomrape plant can produce hundred thousands of seeds; and sixth, the seeds remain viable in the soil for many years and germinate only after stimulation by root exudates of the host.

Some chemical strategies of control are being developed (Jurado-Expósito *et al.*, 1996; Jacobsohn *et al.*, 1998), but breeding for resistance is still the most economic, feasible, and environmentally friendly method of control. Useful levels of resistance have been found in several host parasite systems such as *Sorghum / Striga*, sunflower, and tomato / *O. cernua*, tomato, rapeseed and mustard / *O. aegyptica*, tobacco/ *O. ramosa*, faba bean and vetches / *O. crenata*, among others (see Cubero, 1991, for a review) but no sources of resistance had been described in the genus *Pisum*. We detected sources of resistance in accessions of *P. sativum*, *P. abyssinicum*, *P. arvense*, *P. elatius* and *P. fulvum* (Rubiales *et al.*, 1998) (Fig. 2) and a crossing programme has been started to exploit that resistance in pea breeding.

The possible existence of races or biotypes of *O. crenata* was studied by Cubero and Moreno (1979) and Radwan *et al.* (1988). They found very low level of host/parasite interaction. Differences in the level of agressiveness among populations have been detected (Verkleij and Pieterse, 1994). Molecular analysis suggest that most of the intraespecific variation in *O. crenata* is among individuals and not among hosts nor between regions (Paran *et al.*, 1997; Zeid *et al.*, 1997; Román and Rubiales, 1999) what supports the lack of physiological races and the high flow among broomrape populations. New parasitic biotypes can originate, however, as *O. crenata* populations are very heterogeneous chromosomically (Cubero et al., 1979) as well as genetically (Verkleij and Pieterse, 1994). Thus the introduction of resistant genes might select more virulent variants that could be already present in the heterogeneous broomrape populations, or that could evolve by sexual crossing or mutation. In fact a new race of *O. crenata* has been detected in Israel attacking resistant vetches (Joel, 1997).

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UNDERSTANDING THE BIOLOGY OF BROOMRAPE IS REQUIRED FOR MANIPULATION OF HOST RESISTANCES

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Introduction.

In the Mediterranean area the most damaging parasitic weeds are the broomrapes (*Orobanche* spp.), obligate root parasites that attack most vegetable crops, grain legumes and sunflower. There is so far no economical, feasible or universal mean for the control of any of the seven weedy species of *Orobanche*.

Orobanche does not develop normal roots, is devoid of leaves, and lacks the ability to photosynthesize. The growth of *Orobanche* includes a series of metabolic and developmental steps, each crucial for the establishment of a direct connection with the host and for the survival of the parasite. Some developments, in particular the formation of a haustorium, are common only to parasitic plants.

Each developmental stage can serve as a target for control because it is crucial for the growth and development of the parasite. Hence the understanding of metabolic and developmental aspects of the parasite is essential for any effort to develop effective control measures that will specifically prevent the damage it causes in agricultural fields.

The key steps in broomrape development are described in the following, discussing the vulnerability of each step as a target for control, and presenting examples of the use of developmental knowledge for the control of the parasite.

Orobanche life cycle.

Orobanche exhibits two main life phases: (a) the independent life phase, (b) the parasitic life phase (Joel *et al.* 1995b). The independent phase begins with germination and lasts a few days until the parasite finds a host and develops a haustorium, the feeding organ that connects it to the conductive tissues of the host.

When the seed germinates only a radicle emerges out of the seed coat. The radicle elongates and orients itself towards the host root. The radicle can grow only to a limited extent (a few millimeters), depending on the available resources in the tiny seed. Radicular elongation stops when a host is reached and a haustorium is formed. This independent life phase is entirely facilitated by the consumption of material stored in the seed.

The parasitic life phase starts as soon as a haustorium has developed and nutrients can be derived from the host. Intrusive cells of the haustorium penetrate the host root, eventually forming a physiological bridge between the vascular system of host and that of the parasite (Kuijt, 1969; Losner-Goshen *et al.* 1998). Subsequently the parasite develops a shoot that emerges from soil, flowers and sets seeds.

Seed conditioning and germination.

A chemical stimulus is needed in order to trigger the germination of *Orobanche*, but in order to render the imbibed seed responsive to germination stimulants, a moist environment is required for several days together with suitable temperatures (Joel *et al.* 1995b). This preparatory phase is known as 'conditioning'. The conditioned seeds are quiescent (but active metabolically) until germination is elicited by host root exudates. During conditioning major metabolic pathways are operating in the seed. The rate of respiration changes during the conditioning phase (Bar-Nun and Mayer ,1993) indicating that very active metabolic changes are necessary for the preparation of the seeds for germination.

Gibberellin (GA), if applied during conditioning, reduces the minimum effective exposure time to the germination stimulant (Joel *et al.* 1991), increases the rate of respiratory uptake of oxygen (Bar-Nun and Mayer, 1993) and often causes a noticeable increase in subsequent germination percentage, which supports the assumption that synthesis of GAs occurs during conditioning. Application of inhibitors of GA synthesis to *Orobanche* seeds during conditioning reduces their response to germination stimulants and can prevent germination (Joel *et al.*, 1991, 1995b). Based on these findings we examined the possibility to control the parasite by soil application of uniconazole, a triazole that is commercially used for growth regulation. Application of this inhibitor of gibberellin biosynthesis significantly reduced the number of broomrape infections on sunflower and allowed the development of normal sunflower heads, compared to non-treated controls that were severely affected by broomrape,

with ca. 50% yield loss. These results demonstrate the vulnerability of seed conditioning, and mark it as a possible target for future broomrape control.

Another possible target in the life cycle of broomrapes is germination itself. We know that conditioned *Orobanche* seeds germinate in response to very low concentrations of stimulants. At the same time higher stimulant concentrations inhibit *Orobanche* seed germination (Joel *et al.* 1995b). Exploiting this knowledge, two possible strategies can be adopted to limit *Orobanche* infection by manipulation of the stimulants biosynthesis in host plants:

a. Inhibition of stimulant synthesis, to prevent *Orobanche* germination near crop roots.

b. Over-expression of the stimulant. In this latter case high stimulant concentrations would develop in the immediate vicinity of host roots, suppressing germination rather than triggering it, while further away from the roots, where a parasite seedling cannot reach a host roots, optimal concentrations of the stimulant would prevail resulting in suicidal germination.

Since the first strategy can easily be bypassed in agricultural fields by stimulants originating from neighbouring weeds, we find the latter strategy more promising for effective prevention of parasitism together with depletion of the soil from *Orobanche* seeds bank.

Over-expression of the stimulant should be achieved either by classical breeding or by genetic engineering.

Attachment and penetration.

Attachment of the parasite to host root surface takes place as soon as the parasite meets a host root. This is facilitated by the secretion of an adhesive substance by the parasite (Joel and Losner-Goshen 1994). This step is unlikely to be controlled as long as nothing is known about its control, and as long as there are no indications of possible environmental influences on this process. On the other hand, penetration of *Orobanche* intrusive cells into host tissues seems to be a highly vulnerable step that may be blocked, as already shown in some known *Orobanche* resistances. This is the first stage of intimate contact between cells of host and parasite. This is also the beginning of the true parasitic phase in which the parasite takes nutrients and

water from the host. Therefore it is crucial to further development of the parasite. At this stage *Orobanche* does not behave as a compatible partner in host tissues and there is no coordination with the host during invasion (Joel and Portnoy 1998).

Breeding resistant host genotypes that block the penetration of *Orobanche* haustoria has been one of the most promising approaches to reducing losses due to infestation by the parasite. There are presently some *Orobanche* resistant cultivars of faba bean, sunflower, pepper and vetch (Cubero, 1986, 1991; Goldwasser *et al.* 1996; Kleifeld *et al.* 1996). However, many resistances were lost due to selection in the *Orobanche* populations towards more aggressive biotypes adapted to the newly introduced cultivars (Cubero 1991). In Israel we recently found a new race of *O. crenata* Forssk. that successfully attacks the resistant vetch varieties (Joel and Portnoy 1997).

A way that may overcome this difficulty is engineering *Orobanche* resistance with genes that will neutralize key developmental activities in the parasite, or genes encoding the synthesis of anti-*Orobanche* toxins. These genes should have appropriate promoters that will allow their expression at the infection sites in root tissues. We have already suggested one such promoter when demonstrating the expression of the promoter of a PR protein in *Orobanche* infected transgenic tobacco (Joel and Losner-Goshen 1994, Joel *et al.* 1998). This and similar promoters (Westwood *et al.* 1998) may serve as a powerful tool in the creation of artificial resistances.

Penetration is feasible by a combination of mechanical forces exerted by the haustorium and enzymatic activities that change wall composition in host tissues (Joel and Losner-Goshen 1994). Both mechanisms may be blocked in resistant roots. Mechanical forces may be met by mechanical strengthening of host tissues, such as extra lignification of certain root cell layers. Known enzymatic activities of the parasite may be met by specific mechanisms aimed to neutralize their activity.

The involvement of pectinases was recently proven in an immunocytochemical study that showed in situ presence of pectin methyl esterase in intrusive cells and neighbouring host apoplast, and demonstrated changes in host cell wall pectins that correspond to pectinase activity (Losner-Goshen *et al.* 1998). Other wall degrading enzymes, like polygalacturonase and cutinase, were also found to be active in this system (Joel *et al.* 1998). These mechanisms should serve as specific targets for the inhibition of *Orobanche* development in host tissues.

Resistant crops can also be obtained by grafting. Recent experiments with sunflower gave full protection to susceptible varieties by the use of resistant rootstock.

All non-grafted plants of the resistant varieties were not infected by *O. cumana*, and almost all sunflower plants of the susceptible variety were heavily infected by the parasite. Similar results were respectively obtained with the self-grafted plants, where susceptibility and resistance were maintained. Cross grafting of resistant and susceptible plants always resulted with the same outcome: grafted plants that possessed a rootstock originating from a resistant variety were not infected by *Orobanche*, indicating that the nature of the rootstock determined root resistance. Grafting with resistant rootstock lead to normal development and yield in the resulting sunflower plants. This method, that cuts short the time needed for the development of resistant plants with proven yield qualities, should therefore be seriously considered as a short-term solution for the broomrape problem in some planted crops.

The mature parasite.

After the establishment of a conductive connection between host and parasite the parasite develops a tubercle that accumulates nutrients of host origin. This tubercle is the juvenile parasite. At a certain stage it matures and forms a flowering shoot that emerges above soil surface and produces flowers and seeds.

The development of both the juvenile and the mature parasites is coordinated with that of the host. Cambial activities of host and parasite, for example, are aligned and result in the formation of continuous vessels that bridge between the two. At this stage one can physiologically regard the parasite as an integral part of the host, competing on host resources like a host organ. A presumably possible way to prevent the damage caused by the parasite is helping the host to successfully compete with the parasite with hormonal treatment applied to host shoots.

The mature haustorium provides a direct connection between the conductive tissues of the host and those of the parasite. This route can be exploited and serve for transportation of herbicides to the parasite that develops underground and cannot directly be reached at early stages of its development. Being a strong "sink" for plant metabolites the parasite accumulates toxic levels of systemic herbicides as well. The use of transgenic crops engineered with target-site herbicide-resistances is therefore a most promising solutions for *Orobanche* infestation in many crops, and can fully control the parasite without affecting the crop and its yield (Joel *et al.*, 1995a, Surov *et al.* 1998).

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Westwood et al., 1998

MOLECULAR ANALYSIS OF *Orobanche crenata* POPULATIONS FROM SOUTHERN SPAIN

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Introduction.

Characterisation of genetic diversity is a main goal in population and evolutionary genetic studies as genetic variability provides the basis for evolutionary changes and is a mayor tool for selection and breeding. Studies of morphological and physiological traits have assessed traditionally genetic diversity in plants. Since these characters are likely to be influenced by the environment, alternative methods have been employed to assess genetic variation. Over the years the methods for detecting and analysing genetic diversity have gradually progressed from Mendelian analyses to electroforetic assays of biochemical variants and recently molecular DNA variation between individuals.

Morphological and molecular markers have extensively been used to study this diversity in many organisms, however in the case of *Orobanche* the use of morphological markers to study diversity is quite difficult (Musselman, 1994). The inherent morphological variability within plant populations and the non-photosynthetic nature (no leaves and only short abnormal root) are some disadvantages when working with morphological markers in *Orobanche* studies. The host also may influence the morphology of the plant.

Isozymes have been also used in *Orobanche* diversity studies (Verkleij *et al.*, 1991, Castejón-Muñoz *et al.*, 1991) but they present low levels of polymorphism and as proteins, they are products of gene expression and vary in different tissues, developmental stages and environments. An example of this environmental influence is the study of Verkleij *et al.* (1991) that attribute the higher genetic isoenzimatic variability found in Spanish *Orobanche crenata* populations when compared with populations from Syria, to the fact that the former were taken under field conditions and the second in a glasshouse.

The development of Random Amplified Polymorphic DNA (RAPD) markers has provided a powerful tool for the investigation of genetic diversity. The RAPD procedure requires only small amounts of DNA, and is simpler, less costly, and less labour intensive than other DNA markers methodologies. RAPDs method offers a rapid way to resolve a vast number of discriminatory bands. Since a great number of primers can be assayed and several DNA bands can be differentiated, the number of possible combinations is very high.

The aim of the present study was to determine genetic relationships among field populations of *Orobanche crenata* growing on broad bean in Andalusia (Spain) as the survey and understanding of broomrape population evolution is of main importance to develop adequate breeding programs. Ten individuals per each of the six populations considered in the study were RAPD analysed and pair-wise comparisons of genotypes were used to obtain genetic distances.

MATERIAL AND METHODS.

Material.

Six *Orobanche crenata* populations from southern Spain were used in the present study. Ten broomrape plants were collected from six naturally infested cultivated crops in different fields of Andalusia from May to June, 1998. Freshly floral buds were stored at –80ºC until used.

DNA extraction and Amplification.

Floral buds were used for DNA extraction using the method proposed by Lassner *et al.* (1989), modified by Torres *et al.* (1993). DNA from individual plants was used as a template for PCR-amplification using a Perkin-Elmer Cetus Thermocycler. Twenty-three ten-mer oligonucleotide primers provided by Operon Technologies (Alameda, CA) (table 1) were used. PCR products were separated on a 2% agarose gel in TBE buffer, stained with ethidium bromide and photographed under UV light. Only bands that were clear and reproducible were included in the analysis.

Table 1. RAPD primers analysed:

Primer	Sequence (5'-3')	Primer	Sequence (5'-3')
OPB-03	CATCCCCCTG	OPD-02	GGACCCAACC
OPE-17	CTACTGCCGT	OPG-07	GAACCTGCGG
OPI-16	TCTCCGCCCT	OPJ-01	CCCGGCATAA
OPG-13	CCACACTACC	OPJ-20	AAGCGGCCTC
OPP-09	GTGGTCCGCA	OPS-04	CACCCCCTTG
OPU-09	CCACATCGGT	OPU-11	AGACCCAGAG
OPV-09	TGTACCCGTC	OPAA-7	CTACGCTCAC
OPAB-4	GGCACGCGTT	OPAB-07	GTAAACCGCC
OPAH-04	CTCCCCAGAC	OPAH-13	TGAGTCCGCA
OPAG-4	GGAGCGTACT	MER-02	GTTAGGTCGT
MER-04	GTCCCGTTAC	MER-06	GGTGATGTCC
MER-07	GGGTTGCCGT		

Data analysis.

Polymorphic RAPD bands were treated as binary (presence/absence) characters. Pair-wise comparisons of genotypes were used to obtain genetic distances with Jaccard (1908) and Simple Matching indexes. These similarity coefficients were then used to construct a dendogram by UPGM (Unweighted Pair-Group Method with arithmetical averages) using the Hierarchical Cluster Analysis provided by SYSTAT Software. These two coefficients are recommended when working with qualitative binary data and they have been used in previous intra and interspecific variability studies with RAPD markers (Stiles *et al.*, 1993; Shah *et al.*, 1994; Wu and Lin, 1994; Jain *et al.*, 1994; Karihaloo *et al.*, 1995; Millán *et al.*, 1996)

RESULTS.

Amplification reaction.

DNA extracted was of high quality and PCR amplification reactions gave clear and readable bands. With the 23 primers analysed, we obtained 121 amplified fragments from which 64 bands were polymorphic. From the primers analysed 91% were polymorphic and the number of bands per primer varied from 2 to 9 with a ratio of

5.26 bands/primer. The number of polymorphic bands per primer ranged from 1 to 7 being the level of polymorphism found of 52.9%.

Dendograms and Genetic Distances (GD).

Interpopulation variability

Groups of individuals obtained with the coefficients used, Jaccard and Simple Matching, did not shown significant differences. They gave identical dendograms and similar genetic distances being that provided by Jaccard index slightly higher than that provided by Simple Matching one (Jaccard max. GD of 0.44 and Simple Matching max. GD of 0.37).

Simple Matching index considers the double absence of a band as a similarity. Its utilisation could give wrong interpretations if a double absence of a band does not represent an identity. However, when the identity probability of the sequence in a certain locus randomly chosen is high, for example when working with intraspecific comparations, a double absence of a band can be considered as an identity. According to Skroch *et al.*, (1992) a good indicator of this probability is a high ratio between monomorphic markers and total number of bands. In the present work this ratio was high (0.47), so the use of this coefficient is justified. Therefore, similarity estimations are lower with the Jaccard index than with the other one due to Jaccard coefficient does not consider double absences as similarities (Belaj, 1998).

In the total dendogram obtained with the sixty individuals, the maximum genetic distance found was 0.44 (56% of similarity). Five individuals from one of the populations seemed to be identical with genetic distances of 0, having the same banding pattern. A higher number of markers is needed in order to determine whether they are really identical or not.

Intrapopulation variability.

Although maximum genetic distances found in the total of populations analysed were quite similar, around 0.4, differences were greater attending to minimum values (from 0 to 0.33).

Population	Genetic Distances
Jerez (Cádiz)	0.21 – 0.44
Carmona (Sevilla)	0.00 – 0.41
Mengibar (Jaén)	0.33 – 0.42
Pinos Puente (Granada)	0.29 – 0.44
Purchil (Granada)	0.09 – 0.40
Córdoba	0.33 – 0.40

DISCUSSION.

This study detects little genetic differentiation among populations but a considerable variation among individual broomrape plants within each population. This lack of divergence between populations of *O. crenata* in southern Spain can be explained by a high gene flow between population helped by an efficient dispersal of parasitic seed as well as by the matting system. They are also easily dispersed by humans, machinery, animals and wind.

Our results are different from those found in *Orobanche cumana* (Gagne *et al.*, 1998) where they obtained high differentiation between populations and low intrapopulation genetic diversity suggesting that *O. cumana* might be a self-pollinated species. The degree of variability among individuals in the present study is similar to that found in previous studies with outcrossing species. Huff *et al.* (1993) analysed RAPD variation within and among natural populations of *Buchloë dactyloides*, an outcrossing species, finding that most of the genetic diversity was attributable to differences among individuals within a population.

Low level of inter-population variation has been found in previous studies of *O. crenata* variability (Verkleij *et al.*, 1989; Paran *et al.*, 1997), which is to be expected from its predominantly outcrossing behaviour (Musselman, 1986). Zeid *et al.* (1997) have found extremely low genetic distances between three different *O. crenata* populations from Egypt that have identical banding pattern with a similarity index of 1. DNA used in that study was isolated and purified from 0.2 gr. of *Orobanche* seeds. In this case some absent bands can be hidden by the presence of the same band in another seed genotype from the mixture. In order not to overestimate similarities in population studies it is recommended work with DNA from individual plants.

RAPD´s markers have shown to be very useful in *Orobanche* phylogenetic studies (Paran *et al.*, 1996; Katzir *et al.*, 1996) as well as in population studies (Zeid *et al.*, 1997; Paran *et al.*, 1996; Joel *et al.*, 1998). The use of RAPD followed by Southern hybridisation that detects similarity not only in molecular size but also in molecular sequences (Portnoy *et al.*, 1997), and also the application of SCARS (Sequence Characterise Amplified DNA Regions) (Portnoy *et al.*, 1998; Joel *et al.*, 1998), have been used as an *Orobanche* diagnostic tool. DNA used in these diagnosis studies can be extracted from single *Orobanche* seeds (Joel *et al.*, 1996).

Radwan et al. (1988) showed that diversity of parasitic races may affect the performance of resistant genotypes in a study of variation among geographic accessions of *Orobanche crenata.* The question of whether variation in aggressiveness is genetically determined or it's only a result of variation in seed viability or dormancy induced by environmental conditions affecting different populations, may be answered based on molecular studies (Zeid *et al.*, 1997). Gagne *et al.* (1998), working with molecular markers in *Orobanche cumana* affirm that this approach may be an efficient method for identification of pathogenicity groups in the parasite. The relationship between molecular genetic diversity and the virulence of individuals from each population should allow us to characterise races instead of populations and eventually to obtain molecular markers for these broomrape races.

Cubero and Moreno (1979) and Radwan (1988) found very low level of host-parasite interaction not supporting the existence of races of *O. crenata.* Our study confirm the lack of population diversification. However, differences in the level of aggressiveness among populations have been proposed by Verkleij and Pieterse (1994) suggesting that comparative sutdies have to be carried out with biotypes of *Orobanche* in natural vegetation for a better understanding of the evolution from wild parasitic plants into aggressive parasitic weeds. According to this, cluster analysis of RAPD data has shown high similarities between young *Orobanche aegyptiaca* populations form recently infested fields and known population of the parasite in Israel (Joel *et al.*, 1998) suggesting the possible origin of new infestation. A new race of *O. crenata* attacking resistant vetches has been found in Israel (Joel, 1999). For further studies it also can be considered the partition of variation between populations as a function of more distant geographic origins.

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VIFOR - A SIMULATION MODEL THAT AIDS MANAGEMENT DECISIONS FOR *Orobanche* CONTROL

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Introduction.

Crop-weed competition models are useful tools to quantify the effects of weeds on crop performance under different environmental conditions and to improve both tactical and strategic weed management options (Spitters, 1989; Kropff and Lotz, 1992).

For *O. crenata*, two modelling approaches may be distinguished. The first approach deals with predicting of the development of the *O. crenata* seedbank in the soil (López-Granados and García-Torres, 1993; Schnell *et al.*, 1996; López-Granados and García-Torres, 1997). Estimation of the effect of *O. crenata* infestation on crop growth and yield loss within one growing season is the subject of the second approach. Models of this type are based on simple and static regression equations relating crop yield loss either to the number of parasite seeds found in the soil prior to crop planting (Bernhard *et al.*, 1998) or to the number of emerged *O. crenata* plants at faba bean harvest (Mesa-García and García-Torres, 1984; Linke *et al.*, 1991). These models are not capable of predicting the results of complex interactions between faba bean plants, *O. crenata*, agro-environment, and management practices, and can not be extrapolated to other, as yet unstudied, field situations. The only exception is a dynamic simulation model developed by Kropff and Schippers (1986). This model assumes that *O. crenata* plants act as an extra strong sink for assimilates, and simulates, based on a given number of *O. crenata* plants per faba bean (input variable), the growth and development of both host plant and *O. crenata*. The model, however, does not attempt to predict the level of *O. crenata* infestation in the light of the parasite seedbank in the soil and faba bean root system development.

In this paper we present the structure and application of a Vicia Faba - *Orobanche crenata* model (VIFOR). The development of the model was based on data from both our previous studies (Manschadi *et al.*, 1996; 1997; 1998a-b) and the literature. The VIFOR model allows dynamic simulation of the host-parasite system and estimation of yield losses of faba bean attributable to *O. crenata*, in dependence of genetic, management and environmental factors.

Description of the VIFOR model.

The framework of VIFOR is presented in Fig. 1. Model operation requires four separate input files (genetic and management, soil profile, irrigation, and weather files) that have been described in detail previously (Manschadi *et al.* 1998a). After reading the input data and initializing soil and plant parameters, simulation starts at daily intervals, beginning at the day of planting. Each day the weather data are read from a weather file. The actual dynamic section of the model consists of four components: soil water balance, faba bean development and growth of aerial parts, faba bean root system growth, and *O. crenata* development and dry matter accumulation.

The soil water balance section in the VIFOR model simulates surface runoff, infiltration, deep drainage, potential evapotranspiration, actual plant transpiration, soil evaporation, and soil water redistribution (Manschadi *et al.*, 1998a). A soil water deficit factor (SWDF) is computed from the average fraction of available water (FAW) over the whole root zone. The value of SWDF ranges from 0 (no growth) to 1 (no water stress). The effect of water stress on faba bean growth is taken into account by multiplying the value of light use efficiency (LUE) by SWDF.

Simulation of faba bean growth and development is based on the FAGS model (Manschadi *et al.*, 1998a) that simulates various developmental stages of faba bean, such as emergence, flowering, pod-setting and physiological maturity, as well as leaf, branch and pod numbers as a function of both temperature and photoperiod. Dry matter production is a function of light interception and utilization. Newly produced and retranslocated dry matter is partitioned between roots and shoots, vegetative and generative shoot organs as well as leaves and stems. The end of the natural growth period (physiological maturity, black pods) is reached when the leaf area index reaches zero.

The root growth model simulates the depth of rooting and root-length density (RLD, cm roots cm-3 soil) in each soil layer. This is based on dry matter allocation

to the root system, soil water content, genotype-specific rooting characteristics (e.g. maximum root system depth, ratio of root-length to weight at seedling and maturity and root weighting coefficient) and soil physical properties such as bulk density, sand and silt content (Manschadi *et al.*, 1998b).

The *O. crenata* section predicts the dates and numbers of different *O. crenata* development stages (appressorium, tubercle, bud, emerged shoot and maturity) as well as the daily amount of dry matter allocation to the parasites. The thermal time approach is used to predict the dates of occurrence of various developmental stages of *O. crenata*. The development rate of *O. crenata* is assumed to be directly proportional to soil temperature. The developmental stages of *O. crenata* are numbered from 1 to 6 and when the number of degree-days reaches the specific value, the parasite is assumed to have reached the next developmental stage. The number of *O. crenata* attachments is calculated based on the parasite seed density in soil (input variable) and faba bean root-length density in the upper 15 cm soil layer. Simulation of dry matter partitioning to *O. crenata* in VIFOR is based on the assumptions that: (i) *O. crenata* acts just as an additional sink for assimilates without influencing the host metabolism (Borg, 1986; Manschadi *et al.*, 1996; Manschadi *et al.*, 1997); (ii) the parasites accumulate dry matter after having reached the bud stage; and (iii) following the emergence of the first parasite shoot subsequent attachments will receive no assimilates. Detailed description of this section is reported by Manschadi (1999).

Application of VIFOR model.

The VIFOR model presented here is a useful tool to estimate the growth and yield loss of faba bean crops infested with *O. crenata*. Comparisons between model simulations and the measured data from our field experiments revealed that the VIFOR model was capable of predicting the growth and yield loss of infected faba bean plants, as well as the dry weights of *O. crenata* at various parasite infestation levels and faba bean sowing dates, as well as under different water supply conditions.

The model has been developed using mainly the experimental data of an *O. crenata*-susceptible faba bean cultivar grown in North-West Syria, and calibrated with data from one field experiment only. Therefore, in order to evaluate whether the description of the physiological relationships and the assumptions on which the model is based are realistic, the model needs to be tested with independent data from various cultivars and locations.

The validated model can then be used, particularly in an integrated *O. crenata* control approach, for assisting the management decisions such as: (i) choice of faba bean cultivar to be grown in specific areas; (ii) determination of optimum crop sowing date in order to obtain maximum reduction in *O. crenata* infestation and, at the same time, minimum yield loss of faba bean; (iii) predicting the occurrence of *O. crenata* development stages for the timing of herbicide applications, as it has been reported that the efficiency of this treatment depends mainly on the time of application, i.e. at the tubercle and bud stage (Sauerborn *et al.*, 1989); and (iii) determination of optimal irrigation management (both timing and amount) at a given site. Furthermore, on a large scale, the VIFOR model can be used, by coupling it with Geographic Information Systems (GIS) and Spatial Weather Generator (Göbel *et al.*, 1995), to delineate areas where the parasite poses an actual or potential risk to faba bean cultivation.

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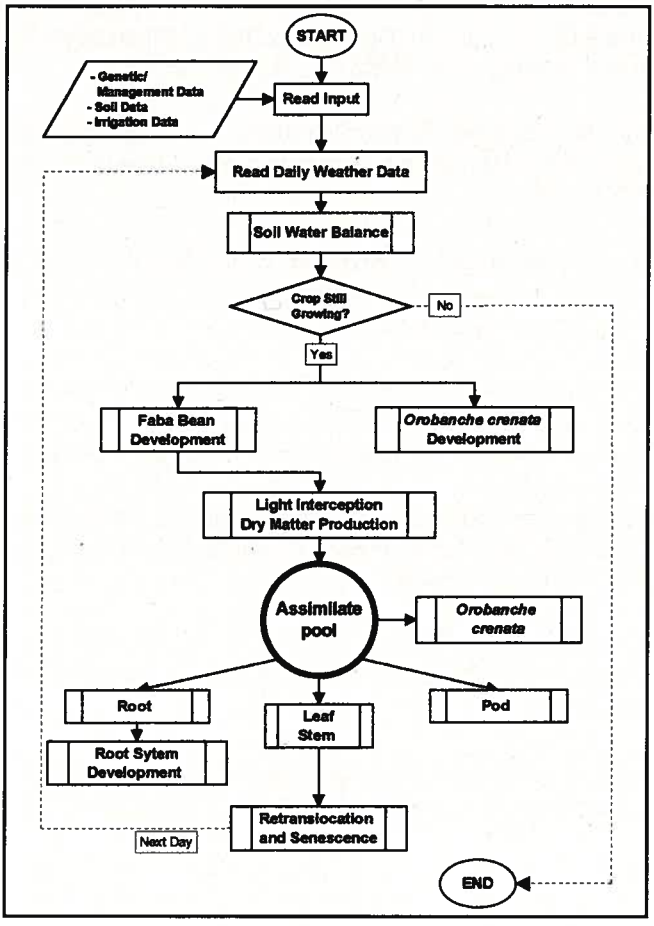

Fig. 1: Flow diagram of the VIFOR model

INHERITANCE OF THE RESISTANCE TO *Orobanche cumana* WALLR. IN SUNFLOWER: A REVIEW

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Although there has not been many studies on the inheritance of the resistance to broomrape in sunflower, those carried out in the former Soviet Union, where breeding of sunflower was very active during the first 70 years of this century, have been published in Russian journals in the Russian language, and very few of them were translated into other western readable languages, so, in most of the cases, the references we have, are those done by some eastern European researchers who could read the Russian language and referenced those works in papers published in western language journals. Therefore, in some of the theories, opinions, and conclusions we have trusted on these references given by prestigious Romanian, Bulgarian and Yugoslavian researchers.

It is world wide known that most of the pioneer breeding work in sunflower was done in the former Soviet Union, mostly in Krasnodar, were Pustovoit and his team, first, and all his disciples and collaborators, later, carried out the development of the sunflower open pollinated varieties which were grown for a long period of time in this country, where sunflower cropped area, in some years, accounted for more than 8.000.000 Has.

Thus, the first references of broomrape attacking sunflower was done there, in the thirties, and the term 'race', appeared for the first time. That broomrape attacking the old Russian sunflower cultivars was considered as belonging to race 'A'. Few years later the first cultivars resistant to race A appeared and the major gene Or1 (dominant) was considered as the responsible for conferring resistance to this race of broomrape. The varieties Kruglik A-41, Saratowskt 169 and Fuxinka-3, etc., were released in 1930. All of them carried resistance to race 'A'. In 1935 race 'B' was already present in the U.S.S.R., and the varieties Jdanov 8281 and Jdanov 8885 were developed, carrying resistance to both races 'A' and 'B'.

After World War II, Pustovoit in Krasnodar developed all the varieties of the VNIIMK and Armavirsky groups as well as the varieties Peredovick and Smena, very well know and cropped all around the world during a long period of time. All of them were resistant to races 'A' and 'B' of *Orobanche curnana* Wallr.

During the sixties the variety VNI1MK 8931, resistant to race 'A' and 'B' started to present severe broomrape attacks in the USSR and Romania, which was a clear symptoms of the appearance of a new race: 'C'. The variety Record, developed in Romania, resisted this new race.

During the seventies, both, cytoplasmic male sterility as well as fertility restoration were discovered in sunflower, thus, the scheme of hybrid seed production was set up. Pure line breeding was then implemented in most of the private and public sunflower breeding programs. Selection of inbred lines resistant to the new races of broomrape that appeared in the U.S.S.R. and Romania was carried out rather than breeding for new resistant o.p. varieties. Hence, to the new race 'C', the Romanian breeders selected resistant lines as S-1358 and O-7586, and to the more virulent race 'E', some lines, as the Romanians P-1380-2 and LC-53 were selected as carrying resistance against this race. Some other lines selected in the region of Odessa (Ukraine), also resulted to be resistant to race 'E'.

During the early nineties, populations of broomrape representing variable, mixtures of most of these races became present in sunflower crops in the south and central parts of Spain. Of particular importance was the presence of race 'E', to which the majority of cultivars cropped in those years were susceptible. In a few years, a complete change in the Spanish sunflower varietal spectrum took place, resulting in a complete renewal of the cultivars, mostly in those areas were race 'E' had become more than a serious problem.

The presence of race 'E' also was noticed in Turkey, where also became a very important problem for sunflower cropping.

Although the fact that the selection carrying resistance to the newest races, been also resistant to the older ones, had been noticed from the very beginning of breeding against broomrape, it was not until 1980 when Vranceanu *et al.* presented a very complete study, depicting the sources of resistance to the prevalent broomrape physiologic races in Romania as well as the type of reaction, number of genes involved and their action.

In Table I, the summarised results of Vranceanu *et al.* (1980) are presented.

The resistance reactions go from R_1 u p to R_5, paralleling the name of the simple major genes (Or_1 to Or_5) conferring resistance to races 'A' through 'E'. Effectively, any of these genes with dominant action, conferred resistance not only to the corresponding race, but also to the older ones. According with the genetic analysis carried out by these researcher, all the. resistance reactions were due to simple dominant genes (Or_1 to Or_5), although since no tests were carried out, allelic relationships were not established, so it was not know whether these genes belonged to the same locus or were independent. Later studies, done by Saavedra et al. (1994) confirmed some of the conclusions reached by Vranceanu et al. (1980), as the reaction of gene Or_5, carried by P-1380-2. Besides the reaction of gene Or_2 in line Jdanov-8281 was also confirmed, in some crosses. A more complex gene model was established with a modifier gene M acting in a epistatic way. It is interesting to notice that these studies were carried out using a broomrape inoculum, mostly attacking confectionery type of sunflower and with a very soft reaction on oil type cultivars (races 'A' and 'B').

Korkhin (1983) found also resistance in a 'recommended Soviet variety' to the race 'B'. When crossed to the susceptible variety Kruglik A-41, the F1 was resistant; although no F2 or BC1 segregation results were presented, he concluded, that this resistance appeared to be controlled by two complementary genes.

Kirichenko *et al.* (1985) carried out a study on the inheritance of resistance to broomrape, using different races of *Orobanche cumana*. In the majority of the cases, a dominant resistant reaction was observed when tests were done with the 'virulent Donestk race'. However, when a resistant line named X502, derived of crosses between *H. tuberosus* and *H. annus*, was crossed to a susceptible line (X1005) the resistance reactions in the segregating generations, were best explained with a model of double recessive epistalisis.

Ish-Shalom-Gordon *et al.* (1993) carried out a very complete study on the inheritance of resistance to sunflower broomrape in Israel, and concluded that in two of the oil type resistant lines studied (SW-501 and RW-637), only a single dominant gene was responsible for the resistance. Nevertheless, no information about which race or population of broomrape was involved in the study was given, although they state that only one strain of broomrape is present in Israel. Hence, it is not possible to assess which gene or genes are present in either two inbred lines.

Domínguez (1996) discovered two dominant independent genes, conferring resistance to a broomrape population in which race 'E' was present, in the restorer oil type line R-41, in Spain. He concluded that one of the genes should be Or_5.

In a very recent work, Sukno *et al.* (1999) studied the inheritance of broomrape resistance in six sunflower inbred lines. All of them presented a monogenic dominant resistance, and after making the proper allelism tests, they did not detect more than one gene, conferring resistance to broomrape in these lines. The inoculum used for these tests was a population in which race 'E' was included. However, when a population in which the new, more virulent, race 'F' was present, only two lines: JD-6 and W-14, showed to be resistant. They concluded that the gene (s) conferring resistance to race 'F' should be either allelic to Or_5 or be present in a locus tightly linked to the Or locus.

In summary, most of the studies carried out on the inheritance of the resistance of sunflower to broomrape, have concluded in a dominant gene action, in most of the cases due to a simple gene, in a low percentage, two or more dominant genes are responsible for the resistance and in very few cases, recessive and, or epistatic action have been described. It is also worth to mention that in those studies where broomrape races have been clearly identified before testing for the inheritance of the resistance to them, the monogenic dominant action has prevailed.

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TABLE1. The array of physiologic races of sunflower broomrape according with Vranceanu *et al.* (1980)

Differential hosts	Broomrape races					Resistance reactions	Resistance genes
	A	B	C	D	E		
AD-66	S	S	S	S	S	R_0	–
Kruglik A-41	R	S	S	S	S	R_1	Or_1
Jdanov 8281	R	R	S	S	S	R_2	Or_2
Record (OPV), H-8280 (IL)	R	R	R	S	S	R_3	Or_3
S-1358, O-7586	R	R	R	R	S	R_4	Or_4
P-1380-2	R	R	R	R	R	R_5	Or_5

R = Resistant
S = Susceptible

RESISTANCE TO *Orobanche* IN SUNFLOWER: MECHANISMS OF RESISTANCE IN THE HOST-PLANT/*Orobanche* SYSTEM

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Introduction.

The genus *Orobanche* has more than 100 species species which infect the roots of many crops of economic importance in the Mediterranean regions, eastern Europe and the former USSR countries. Yield losses are routinely about 50% and can be up to 100% (Parker and Riches, 1993). Control of *Orobanche* species is difficult, because all the stages of parasite development are linked to chemical signals during the life-cycle of the host plant.

The distribution, biology and control of *Orobanche* spp. have been the subject of a number of previous reviews, which have been used as source material for this contribution (Cubero, 1986, 1991; Parker, 1986; Parker and Riches, 1993; Wegmann *et al.*, 1991; Sackston, 1992; Lane *et al.*, 1997). The purpose of this review is to present the most significant results so far achieved in the breeding for resistance to *Orobanche cernua* (*O. cumana*) in sunflower.

Orobanche cernua (*O. cumana*)/Sunflower.

The component of the species complex *Orobanche cernua* which attacks sunflower often referred to as *O. cumana* has been of great importance as pest of sunflower in many countries across the Mediterranean, eastern Europe and the former USSR.

The rapid expansion of the sunflower in Russia by the end of the last century was threaten by the expansion of the broomrape. Thus, the selection of resistant sunflower to *O. cernua/O. cumana*, has been a constant objective of Russian breeding since Pustovoit undertook the task of sunflower breeding in 1910 (Pustovoit, 1966). Selections were initially collected from farmer's fields in the Saratov region, and were initially based on field trials, and since 1921, also on pot tests (Pustovoit, 1973). A range of sunflower populations were developed with complete resistance by 1916.

And by 1925 95% of the crop grown in Pustovoit's region was based on resistant populations (see Pustovoit, 1967, 1976 and Cubero, 1986 and 1991 as valuable reviews). In 1925, susceptibility of these varieties to *O. cumana* was reported from Ukraine and Moldava. Thus, two races were designated as A and B, respectively. Intensive efforts were made to locate resistance to the new race and it was found among a landrace sunflowers collected from farmer's field in Ukraine. The development of new races of the *O. cernua/O. cumana* have been identified in different places. The breeders from different countries have followed the nomenclature started by soviet breeders. Nevertheless, the complexity of this pathogen suggest that what it is known as races C, D, E , etc., in different countries may be different pathotypes from country to country.

In the mid 1960s, a new variant, named C, with virulence on all the resistant varieties was identified in Moldava and Ukraine. This race is prevalent across the sunflower growing regions of the south of former USSR. In 1990 a new race, D, was first identified in the Krasnodar region. It seems that there is not currently an available source of resistance to this new race in the former USSR (Antonova, 1994).

The VNIIMK varieties from the former USSR with resistance to races A and B were resistant to *O. cernua/O. cumana* in Rumania until the mid 1960s except in the south-eastern regions near Moldava. A total of five races were identified using a set of Romanian and former USSR sunflower varieties as differential series, (Vrânceanu *et al.*, 1986). The resistance to the Romanian race D was found in the variety S 1358 and the source of resistance to race E was found in the inbred line P1380. This line has been considered the universal source of resistance until recently.

Sunflower Sources of Resistance to *O. cernua/O. cumana.*

The sources of resistance were originally from within sunflower landraces, but also wild sunflower species have been a valuable source of resistance. The early breeding work included the selection among landraces of cultivated sunflowers (Pustovoit, 1966, 1973, and 1976). Nevertheless, interespecific hybridization has also been a valuable source of *Orobanche* resistance genes in the early history of sunflower breeding and presently. Among the polyploid perennial species, *Helianthus tuberosus* has been the most frequently used, (Pustovoit, 1966; Pogorletskii, 1974; Logvinenko and Logvinenko, 1980 and Kostiuk, 1986; Vrânceanu *et al.*, 1980).

Mechanism of Resistance and Tolerance.

There was very little information available about the crucial subject of mechanisms of resistance and on the genetics of the host/*Orobanche* interactions at the time Cubero (1986 and 1991) made the reviews about breeding for resistance to *Orobanche*. Some recent physiological and biochemical studies are allowing to have some light in this subject. In dealing with *Orobanche* it would be appropriate to define as tolerant those varieties which are parasitized with the same frequency than susceptible varieties but without the yield loss observed in susceptible varieties. The resistant varieties would be those which are not parasitized at all or have significant less frequency of parasites on their roots than susceptible varieties.

Tolerance Mechanisms.

The crops are normally damaged by *Orobanche* spp. mainly because the parasite withdraws the water from the host root. In *O. ramosa*/tobacco and *O. crenata*/faba bean systems it has been shown that in many cases the osmotic values were higher in the *Orobanche* tissue than in the host tissue. Thus, the *Orobanche* plants have higher osmotic pressure compared with it host plant. Sucrose is the main solute absorbed by *Orobanche* from the host root (Whitney, 1972; Aber *et al.*, 1983). The sucrose is cleavaged into glucose and fructose and it doubles the osmotic value of the sugar component. Part of the fructose is transferred into mannitol. Under water stess conditions mannitol becomes more prominent (Wegmann 1986; Harloff and Wegmann 1987; Wegmann *et al.*, 1991). Mannitol, a rare component in higher plants, is a constituent of many *Orobanche* species such as *O. cernua* (Kiesel, 1923; in Wegmann *et al.*, 1991), *O. hederae* and *O. elatior* (Press *et al.*, 1986) and the already mentioned *O. ramosa* and *O. crenata* (Wegmann *et al.*, 1991).

The higher osmotic adjustment of the host plant could be the basis for tolerance to *Orobanche* in several crops. Thus, the faba bean cultivar G 402, tolerant to *O. crenata,* has higher osmotic values than susceptible faba bean cultivars (Wegmann *et al.*, 1991). The breeding for resistance to *Orobanche* in faba bean have shown than additivity was always very strong (Cubero and Hernandez, 1991). Before the faba bean G 402 cultivar was found, only differences in susceptibility were found (Cubero, 1986 and 1991). Tolerance rather than resistance may be the main difference between faba bean cultivars. Osmotic adjustment is probably a polygenic factor and would explain why it is so difficult to bread for resistance to *Orobanche* in faba bean. The G 402 source of resistance may have both a higher osmotic values than susceptible cultivars as well as other mechanisms of resistance.

Resistance Mechanisms.

The resistance mechanisms have to limits any of the steps from the *Orobanche* seed germination to the successful establishment of a direct connection between host and parasite vessels.

The germination of most *Orobanche* species depends on the presence of chemical inductor which is released by the host root. Strigol which is exuded by cotton roots was the first germination stimulant compound active in *Striga, Orobanche* and *Alectra* (Cook *et al.*, 1972). Yet, none of these parasitic plants can parasitize cotton. Strigol has also been isolated from maize, sorghum and millet, all of them host plants for *Striga* but not for *Orobanche* (Siame *et al.*, 1993). Other *Orobanche* seed germination stimulant include sorgolactone, alectrol and others (Wegmann, 1994). Gibberelins have been reported to increase the activity of germination stimulants on *O. crenata* seeds (Garas *et al.*, 1974). The germination inducer cannot be responsible for the host specificity of *Orobanche* species, as both host and not host plants may induce germination of *Orobanche* seeds. Nevertheless a resistant mechanism could be the result of plants being deficient in the production of the germination stimulant. Certainly a mutation in this sense would most probably be recessive. An example of the importance of root exudates in the amount of parasites per host plant can be illustrated by the work made with pepper as a trap and catch crop for *O. aegyptiaca* and *O. cernua* (Hershenhorn *et al.*, 1996). In this work, tomato roots induced less than 10% *O. aegyptiaca* seed germination but were highly susceptible to the parasite (30 parasites per host plant). More than 50% of Egyptian broomrape seeds germinated in the presence of pepper roots, but only few were able to attach to the roots. Interplanting tomato with pepper induced a fourfold increase in the number of nodding broomrape on tomato roots compared to the number of parasites on tomato roots planted alone. It is therefore possible that differences in resistance between cultivars may be the result of differences in the proportion of *Orobanche* seeds induced to germinate in the soil by the host plants. Unless a variety would completely lack of *Orobanche* seed germination stimulant, this type of resistance only would be useful in soils with little infestation.

During germination the seed of *Orobanche* produce a radicle like organ, the "procaulône" which consist of two parts, the main core and the extremity which lacks the cap and typical meristem of roots. As soon as the tip of the "procaulône" contacts a suitable host root, it enlarges and differentiates papillae. No enlargement is observed in the "procaulône" tips in contact with non host species such as flax (Hameed and Foy, 1991). Some chemical signal must be provided by the host root. The lack

of this signal would result in another resistance mechanisms. If ever found, this mechanism most probably would be recessive.

The host root zone of contact and of penetration of the parasite swellen and became yellowing. One part of the parasite seed will remain at the outside of the root and will produce the tubercle, the other part will form the internal endophyte. A successful implantation of *Orobanche* needs the establishment of connections between the conducting elements of the two plants. Anything preventing this successful implantation would result in a resistance reaction.

Early studies suggested penetration between host cells by enzymatic dissolution of the middle lamella with no damage to cells themselves (Dör and Kollmann, 1974). More recent studies point to more complex process, involving both the dissolution of the middle lamella and mechanical pressure which pushes portions of cell wall side, or extends host cell walls in these regions (Joel and Losner-Goshen, 1994). Nevertheless, the intrusive cells of *Orobanche* push their way between host cells rather than through them. This penetrations is possible due to the activity of enzymes that change the composition and physical properties of the host cell walls and middle lamella in the path of the haustorium. Pectolitic activity by the haustorium of *O. aegyptiaca* (Losner-Goshen et al., 1998) as well as cell wall degrading enzymes such as cellulase and polygalacturonase (Singh and Singh, 1993) may be involved in the haustorium penetration. Phenolic and non-phenolic peroxidase activities may also be involved in the haustorium penetration of the host root (Shomer-Ilan, 1994).

Possible resistance factors during the parasite haustorium penetration of the host root may include mechanical barriers like lignification, phytoalexin formation, false hormone supply and others.

A provisional review and new proposals about possible mechanism of resistance to broomrape in sunflower is presented in Table 1.

The first indication of resistance based on mechanical barriers were reported by Pustovoit who thought that the resistance of sunflower to *O. cernua/O. cumana* was produced by the formation of a callus through swelling in the root and the action of cell juices (Pustovoit, 1928, 1939 in Pustovoit, 1966). Peroxidase excreted by the parasite was suggested to be involved in the protective lignification of the host cells (Panchenko and Antonova, 1974, Antonova, 1976, 1977 and 1978) as resistance reactions in sunflower was accompanied by accumulation of lignin in damaged root cells. More recent studies about the differences in peroxidase production between

races of *O. cernua/O. cumana* has allowed to postulate a possible explanation for several of the gen-for-gen interactions in the sunflower/*O. cernua* system (Antonova and ter Borg, 1996). Russian Race A of *O. cernua/O. cumana* can be regarded as the wild type growing in *Artemisia* spp. and old sunflower cultivars. The first resistance cultivars (Kruglik A41, Saratovskii 169 and others) roots had swellings at the sites of attack. Dead *Orobanche* seedlings were observed in their center, indicating the parasite haustorium could not overcome this barrier. When race B of *O. cernua/O. cumana* attacked these cultivars there was not swelling. The stem tissues of race B has twice the amount of peroxidase activity than stem tissues of race A. (Ukrainsky, 1938 in Antonova and ter Borg 1996). Thus, race B might be more virulent than race A because of its higher peroxidase concentration. The resistant cultivars to race B (Jdanov 8281, VNIIMK 8931, etc.) did not show any swelling. Recent studies with the resistant cultivar Edirne have shown that parasitic cells became encapsulated in the root cortex (Dörr et al., 1994). Necrotic root cells surrounding haustoria in the deep layers of the cortex have been observed (Antonova, 1978; Dyakov and Antonova, 1978). Necrotic cell walls and cytoplasm around the haustoria often show lignification. The assumption made by Antonova and ter Borg (1996) is that the haustoria of race B of *O. cernua/O. cumana* can not cross the cortex. Thus the mechanism of resistance of these cultivars against race B would include a hypersensitive-like reaction of similar characteristics of that occurring when the resistant sunflower root are infected with the obligate parasite *Plasmopara halstedii.* In this case, after the degeneration of cytoplasm of death cells, a formation of a solid mass around the fungal structures also occurred. Antonova (1977) pointed out the similarities in metabolic changes occurring in sunflower cell infested with *P. halstedii* and those caused by *O. cernua/O. cumana.*

The race C of *O. cernua/O. cumana* can cross the cortex and the hypersensitive reaction of cultivars with resistance to race B. This may be done by a higher speed of growth into the host helped by the production of intra and extra-cellular peroxidase. The resistance to race C (present in sunflower cultivars: Odesski 63, Star, Record, Progess, etc.) is controlled by one dominant gene Or_3. It seems that this gene controls the lignin formation in sunflower vessels (Tolmachev, 1991). The mechanism of resistance would include the accumulation of phenolic compounds in damaged cells. The meeting of these phenolic compounds of the host with the extracellular peroxidase of *O. cernua/O. cumana,* could induce the polymerization of the phenolic compounds into lignin. Thus, the lignin production in the infected sunflower roots is induced by external peroxidase (Antonova and ter Borg, 1996). The lignin is deposited rapidly after infection, as a continuos layer before the cells of the haustorium penetrate the vessel. The haustoria may die as a result of lignification of the

host vessels. Antonova and ter Borg (1996) suggest that the more virulent Russian *O. cernua/O. cumana* race D has evolved from race C by reduction of the exudation of peroxidase, while maintaining a strong intracellular peroxidase activity in the apical cells of the "procaulône" and the haustorium. In this way the sunflower Or_3 resistance has been overcome by adaptation of the pathogen. Antonova and ter Borg (1996) did not propose neither a mechanism of resistance for the sunflower genes Or_4 and Or_5, nor the cause of virulence of the *O. cernua/O. cumana* race E.

Wegmann (1986) questioned whether phytoalexins may be involved in Orobanche resistance. Phytoalexins are secondary metabolites of the plant which only are formed when the plant is attacked by fungi, bacteria or viruses. They act as fungicidal or fugistatics; batericidal or bacteriostatic and some times virustatic. It was found that the sunflower leaves produced the phytoalexines scopoletin and ayapin, but only scopoletin was found in the roots at much lower concentration than in the leaves. The infected root tissue of the sunflower resistant cultivar 81-14 produced double as much scopoletin than the susceptible cultivar giganta (V. Elert, 1988; in Wegmann *et al.*, 1991). There was a clear correlation between *Orobanche* resistance and scopoletin production. The scopoletin was toxic for *Orobanche* tissue (submerced microcalli) as an inhibitory effect on the respiratory oxygen uptake (Wegmann et *al.*, 1991). Similarly to this sunflower finging, phytoalexin formation has been shown in chickpea (*Cicer arietinum*) cultivars resistant to *O. crenata* (Stadler, 1990; in Wegmann *et al.*, 1991). The resistant chickpea cultivar ILC 280 produced 28 times as much maakiain than the susceptible cultivar FLIP 81/35W and 8 times as much medicarpin. As in sunflower the capacity of phytoalexin biosynthesis in the roots of chickpea was much lower than in the leaves.

Wegmann *et al.*, (1991), also mentioned that phytoalexin biosynthesis is simply inherited, in many cases monogenic. As it has been mentioned earlier in this review, both genes Or_4 and Or_5, most probably were introduced into sunflower from the perennial species *H. tuberosus.* It could be possible that these genes may govern the phytoalexin synthesis. Different phytoalexins may be involved in the resistance as well as doses effect. Perennial *Helianthus* species are generally resistant or immune to *O. cernua/O. cumana* infections while the annual *Helianthus* species are mostly susceptible (Dominguez *et al.*, 1996). It would be interesting to find out if the perennial *Helianthus* species produce higher amounts of phytoalexins than the annual forms of this genus.

Some times in resistant cultivars, it can happen that the haustorium grows fast and vigorously developing a broomrape suggesting that the resistance in this case

is overcome by the metabolic detoxification of the phytoalexin. Detoxification of scopoletin was demonstrated in submersed cell cultures of *O. ramosa* suggesting that resistance had to be considered a quantitative factor (Wegmann, 1994). The new *O. cernua/O. cumana* race capable of cause severe infections in sunflower hybrids which carry the Or_5 gene, recently found in Spain (Alonso *et al.*, 1996), could be a mutant with high capacity for phytoalexin detoxification, if Or_5 resistance is due to phytoalexin synthesis. Homozygous lines carrying the Or_5 were less infected than hybrids carrying the Or_5 gene in heterozygous condition. Homozygous Or_5Or_5 inbred lines had only 30% of the plants infected with emerged broomrapes and an average of 0.6 broomrapes per host plant. The heterozygous hybrids Or_5or_5 had 11.4 broomrape per host plant and 95% of the plants showed emeged broomrapes. In the root inspections there were also differences. Homozigous Or_5Or_5 showed 55% of the plants with parasites (nodules or emerged broorapes) with an average of 2.1 parasites per host plant. The heterozygous Or_5or_5 had 100% de plants parasitized and 20.4 parasites per host plant (Alonso *et al.*, 1996).

Despite *V. faba, V sativa* and *V. narbonensis* are known to synthesize wyerone and related furanoacetylene phytoalexins in the leaves, these species do not produce them in the roots (Robeson and Harbone, 1989; in Wegmann *et al.*, 1991). The lack of root phytoalexins in *Orobanche* resistant *Vicia* spp. could be the explanation for the difficulty in finding a single gene dominant resistance in these species. The nature of the resistance found in some *Vicia* spp. must be due to different mechanisms of resistance in these species.

The low number of *Orobanche* spikes in the faba bean cultivar G 402 have received several possible explanations such as: low production of lateral roots and high compact root mass (Nassib *et al.*, 1978); deeper growth of the root system (Cubero, 1991); reduced production of germination stimulants (Aalders and Pieters, 1986; Wegmann, 1986) or the development of mechanical and physiological barriers (Nassib *et al.*, 1984; Aalders and Pieters, 1986; Wegmann, 1986). Nevertheless, differences in root mass or root architecture does not look as a major mechanism of resistance. On the other hand, the activity of root exudates as germination stimulants of both resistant and susceptible faba bean cultivars seem to be the same (Khalaf and Bastawesy, 1989). Attia (1992, in Zaitoun and ter Borg, 1994) observed that the root of G 402 have an intact endodermal layer with thick walls due to reduced secondary growth. Zaitoun et al.(1991) reported that microscopic inspection of infected faba bean cultivar G402 roots revealed the development of a corky tissue at the site of penetration and some cavities in the inner most tissues (xylem tissues) acting as a barrier for further establishment of broomrape. This type of resistance mechanism

was absent in susceptible faba bean cultivars. More recently, in a study with resistant faba bean cultivars from Egypt (402/29 and 674/154) and Spain (Baraca), Zaitoun and ter Borg (1994) found that resistant reaction in faba bean included hypersensitivity at the flowering stage and partial resistance at ripening. None of the investigated lines or cultivars was completely resistant and there was plant to plant variation withing cultivars. Different levels of resistance were characterized: The first is a direct reaction leading to necrosis at the site of contact in the very first stage of growth. The hypersensitivity in the faba bean roots and the brownish coloring of the host cells suggests that phenolic compounds were involved (Dör *et al.*, 1994). Also, high amounts of peroxidases in infected faba bean roots have been reported (Kirollos and El-Hafeez, 1985). As mentioned by Antonova (1994), the phenolic compounds are polymerized by peroxidases to form lignin in sunflower/*O. cernua* system. This could be also the case in the faba bean G 402/*O. crenata* interaction. The second is barrier developing in the host root after the formation of a small tubercle. The cork tissue develops from the cortex (Zaitoun *et al.*, 1991). Both the hypersensitivity after just a slight attack and the building of a barrier in a later phase might be due to the same basic mechanism, i.e., the formation of lignin by polymerization of phenolic compounds. Yet, the barrier in the host root of faba bean include corky tissue (Zaitoun *et al.*, 1991), which is based on fatty and waxy components. This would point to another mechanism. The abundance of necrotic lessions at the attachment point of the parasite observed on the roots of purple vetch varieties "Popany" and "Sadot" (Goldwasser *et al.*, 1996 and 1997) suggest a hypersensitivity reaction. This was followed by a secretion of a brownish/red compound which filled the host-parasite interface stopping the haustorium growth. This could be a lignification reaction similar to the one observed in the sunflower/*O. cernua* system

In false host plant such as *Linum usitatissimum*, neither phytoalexins nor compounds toxic to *Orobanche* cell cultures have been found; however, the haustorium died with decoloration (Chen, 1991, in Wegmann, 1994). It was concluded that a false supply of phytohormones from Linum plants does not allow the *Orobanche* to develop.

The knowledge of the mechanism of resistance and immunity not only facilitate the breeding programs, but also provides the basis for gene transfers related to resistance. This knowledge could also help in the integrated control by combining genetic resistance with chemical plant protection. Thus, the application of subtoxic concentrations of certain fungicides or herbicides, which fortify the phytoalexin formation (Cartwright *et al.*, 1977; Grinstein *et al.*, 1981; Grisebach *et al.*, 1982; Kömives and Casida 1983; Ward *et al.*, 1980) could reinforce the effect of a resis-

tance gene responsible for the phytoalexin formation. On the other hand, the herbicide glyphosate, often used against *O. crenata* in faba beans, act suppressing the phytoalexin formation (Keen *et al.*, 1982) and thus would act in the wrong direction. Also, the understanding of mechanisms of resistance could provide a method for reliable, quick, massive and out season screenings for resistance.

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Table 1. Provisional review and proposal of the mechanisms of *O. cernua/O. cumana* virulence and sunflower resistance genes. Adapted from Antonova and ter Borg 1996.

O. cernua/O. cumana			Sunflower	
Race	**Characteristics**	**Gene**	**Resistance mechanism**	**Resistant cultivar type**
A	Wild type on *Artemisia* spp and local sunflower. Slow growth into host	Or_1	Swelling on host root at site of intruding parasite; haustoria die in its centre	Kruglik A-41
B	Double amount of peroxidase in stem	Or_2	No swelling of host root. Dense cell walls underneath epidermis. Hypersensitivity and necrosis in cortex, small amounts of phenolic compounds	Jdanov 8281, VNIIMK 8931, Peredovick
C	High speed of growth into host; Intra and extracellular peroxidase production	Or_3	Increased production of phenolic compounds; lignin precursos in cortex. Lignin formation at site of contact between young haustorium and xylem vessels	Progress, Record, H-8280
Romanian D	Similar to race C. Plus increased spead of grouth?	Or_4	Phytoalexin (scopoletin) production?	S-1358, 0-7586
Romanian E	Similar to race C and D plus higher capacity of detoxification of scopoletin?	Or_5	Higher production of scopoletin?. Synthesis of other phytoalexin?	P-1380
Russian D Spanish G?	Peroxidase not excreted outside the apical cells. Intracellular peroxidase only. Detoxification of scopoletin?	or_6, or_7?. None dominant.	Recesive resitance to race G due to unknown causes	So far no hybrid cultivar developed with resistance.

NEW RACES OF *Orobanche cumana* ON SUNFLOWER

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1º CHRONOLOGY.

* In 1958 was documented the first attack of *Orobanche cumana* on sunflower (confectionery type) in Guadalajara (Central Spain).
* In 1978 first attack on oil crop (Peredovik) in Tarancón - Cuenca
* 1980 first presence in Andalucía (Fuente de Piedra - Málaga)
* 1994 Registration of the first *Orobanche* resistant hybrids.
* 1995 Two small focus of more virulent race appeared on resistant hybrids.
* 1998 Many spots of new virulent races.
* 2000 100% Andalucía affected by normal race of *Orobanche*.
* In Turkey the new race appeared in 1993. In 1998 there were some areas affected
* In Krasnodar - Russia there are also infestations of new races.

2º NORMAL RACE.

Using different tester it is possible to see different proportions and presence or not of the attack. Depending of type of tester you 'find' more or less races. The race we called 'normal' is a mixture of races with lower virulence.

When seeds of *Orobanche* are collected from different areas they are composed of different races in different proportions depending on history of that parcel: origin of the infections, rotations, hybrids planted etc.

3º NEW RACES.

New races overcome all the resistant hybrids to the normal race .We do not yet know exactly how many there are. The severity of the new races is different from one sample to another and from the different countries. Perhaps the new race from Turkey is the most severe.

We found many small spots of susceptible plants in resistant hybrids like a more aggressive descendent of the *Orobanche* plant. The widespread of the new races is related to appearance of new plants in many places, not only coming from the original focus.

4° RESISTANCE.

Resistance to the 'normal' race is easy. There are some genes with dominant character. Of course there are also recessive and modified genes. Today there are many resistant hybrids registered in Spain. Farmers are always using resistant hybrids in affected areas (400,000 Has).

The lines with resistance to a new race must also be resistant to the old ones. In the field and in the test, you cannot separate the *Orobanche* seeds by races as there is always a mixture of old and new races present.

It is more difficult to find resistance to the new races of Spain. The genes are segregating with the resistance to new races and it is difficult to fix that resistance therefore their hybrids are not 100% resistant. It is also very difficult to find any resistance to the Turkish races.

In the lines there is segregation for the resistance to the new races of *Orobanche* but effective selection in the line cannot be done.

Theoretically double resistance due to occurrence in the female and in the restorer should be better than the simple; but it is not clear.

We tested many different lines of wild origin, but all of them were susceptible to the new Turkish race. It is clear that when working with wild germplasm each generation of backcrosses must be tested because the resistance is normally lost in the process.

5° WORKS FOR FUTURE.

* It is very important to increase our knowledge about the mechanism of susceptibility and resistance from the point of view of biochemistry, physiology, etc.

* It could be beneficial to understand why in a pure line there is segregation with the new races.
* It is necessary to find any resistant material to incorporate those genes in commercial hybrids. To achieve this, one must work with wild species, world collections, etc., and also breed with small differences.
* It would be optimal also to have herbicide control as a security measure. The plant could also be resistant to the herbicide.
* Molecular markers.

DEVELOPMENT OF BROOMRAPE RESISTANT SUNFLOWER GERMPLASM UTILIZING WILD *Helianthus* SPECIES

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Introduction.

Sunflower broomrape *(Orobanche cernua* Loefl. syn. *O. cumana* Wallr.) is currently regarded as one of the most important constraints in sunflower (*Helianthus annuus* L.) production in Spain and the regions around the Black Sea (Alonso *et al.*, 1996; Bulbul *et al.*, 1991; Domínguez *et al.*,1996a; Shindrova, 1994). Attacks are frequently severe and yield losses can reach 50 % (Domínguez, 1996).

Each broomrape plant produces thousands of tiny seeds that are easily spread by wind, soil, farm machinery, water and the sunflower achenes. The seeds, outside the host rizosphere may remain dormant and viable for more than ten years. Control measures for broomrape such as solarization and soil fumigation have been described but are prohibitively expensive. Herbicides offer some chemical control (García Torres *et al.*,1988). However, at present, genetic resistance is the most effective and feasible control against *O. cernua*. Genetic resistance was introduced into susceptible sunflower mainly from the wild species H. tuberosus (Pustovoit, 1966). However, the use of resistant cultivars has been frequently followed by the appearance of new pathogenic races overcoming the resistance and there is a continuous need of new sources of resistance. The inheritance of resistance to broomrape was found to be monogenic with five Or genes (Or_1-Or_5) described in five differential lines that provide an accumulative resistance to five successive races, namely A-E (Vranceanu *et al.*, 1986). In Spain, since the 1970´s when broomrape was only present in small areas of confectionery sunflower production fields, the parasite has quickly spread in central and southern areas causing serious infections in oilseed varieties. Racial studies identified races overcoming Or_1, Or_3 and Or_4 genes but not Or_2 and Or_5 (Melero-Vara et al., 1995). Later studies have shown an evolution of the racial situation and a new race, named F, which overcomes all the known genes of resistance, including Or_2 and Or_5, has been identified (Alonso *et al.*, 1996; Domínguez *et al.*, 1996a).

Sources of resistance

Sources of resistance to the recent virulent races found in cultivated germplasm have been scant. Gulya *et al.* (1994), found only 22 resistant entries in a field evaluation of 903 accessions in Turkey. Domínguez *et al.* (1996b) evaluated a total of 429 accessions of different origins and found only 8 resistant and 33 segregating for resistance to race E. In a more recent evaluation only 4 entries out of 55, previously reported as resistant in Turkey (Gulya *et al.*, 1994), were found resistant against race F (Fernández-Martínez *et al.*, 1999). In contrast, a high level of resistance has been found in wild Helianthus species. In Spain, Ruso *et al.* (1996) and Fernández-Martínez *et al.* (1999) found resistance to several virulent races, included race F, in 29 perennial wild species. The annual species showed a much lower level of resistance with only four out of 18 entries evaluated, showing resistance to race F (Fernández-Martínez *et al.*,1999). Resistance to *O. cumana* has also been identified in wild Helianthus species in other countries (Korell *et al.*, 1996; Christov *et al.*, 1996; Skoric, 1988).

Transfer of resistance to cultivated material.

In general the resistance to *O. cernua* found in annual sunflower species can be easily transferred to cultivated material but, in spite of the high level of resistance found in the perennial wild species, the majority are not suitable for breeding purposes, because of interspecific incompatibility, postzygotic abortion of the hybrids and F1 sterility. Therefore, although interspecific transfer of bromrape resistance from H. tuberosus was achieved in early breeding work in the former USSR (Pustovoit, 1966), in most cases this resistance has remained unexploited.

However, there are more recent reports in several countries, on the development of cultivated germplasm incorporating resistance to *O.cernua* from wild species. The species from which this resistance has been transferred are: *H. maximiliani, H. mollis, H. pauciflorus* and *H. divaricatus* in the former USSR (Porgoriesky and Geshele,1976), *H. pauciflorus, H. decapetalus, H. tuberosus* and *H. argophylus* in Bulgaria (Christoff et al.,1996) and *H. tuberosus, H. glaucophylus , H. resinosus* and *H. debilis* in Yugoslavia (Skoric, 1992).

In Spain, a breeding programme to transfer *O. cernua* resistance from wild resistant species to cultivated sunflower has been carried out since 1994 at the Institute of Sustainable Agriculture, Córdoba, in collaboration with Dr. C.C. Jan from USDA-ARS

Fargo, North Dacota,USA, who produced the most difficult interspecific hybrids with perennial species. Three annual and twelve perennial species, with three ploidy levels: diploids (2n=34), tetraploids (2n=68) and hexaploids (2n=102), showing resistance to race E, and some also to race F, were used. Special techniques such as embryo rescue to overcome abortion of F1 hybrids, and chromosome doubling to improve the fertility of interspecific hybrids (Jan,1997) were applied The hexaploid wild x cultivated hybrids, do not need chromosome doubling and their BC_1F_1 and BC_2F_1 were produced and screened for broomrape resistance. For tetraploids and diploid species, F_1 hybrids were obtained and treated with colchicine to restore fertility and the chromosomally doubled heads were sib pollinated to produce amphiploids. The amphiploids of tetraploids perennials with cultivated material were tested for broomrape resistance and resistant plants were backcrossed three times to cultivated lines. Plants of generations BC_1F_1 (2n=68),BC_2F_1 (2n=51) and BC_3F_1 (2n=34 to 51) were screened for resistance and resistant plants with 34 chromosomes were obtained. For the amphiploids of diploid x cultivated material, resistant amphiploid plants were backcrossed twice to cultivated lines and plants of BC_1F_1 (2n=51) and BC_2F_1 (2n=34 to 51) were evaluated for broomrape resistance and BC_2 resistant plants with 34 chromosomes were selected. The annual species (2n=34) did not need special techniques. BC_1F_1 resistant plants, (cultivated x wild) x cultivated, were selected and selfed during several generations to obtain lines breeding true for resistance.

The following resistant derived material has been developed in this programme:

– BC_1F_4 lines, derived from *H. anomalus* and *H. exilis*, breeding true for resistance to races E and F and BC_1F_1 resistant plants of the cross, (cultivated x *H. debilis*) x cultivated.

– BC_1F_1 and BC_2F_1 progenies of the diploid species *H. giganteus* and the hexaploids *H. laevigatus, H. resinosus* and *H. pauciflorus* resistant to race E (Sukno et *al.*, 1998).

– BC_1F_1 and BC_2F_1 progenies of the diploid perennials, *H. angustifolius, H. cusickii, H. divaricatus, H. grosseseratus, H. maximiliani* and *H. nuttallii* and the tetraploids *H. hirsutus* and *H. stromosus*, segregating for resistance to several virulent races (Jan et *al.*, 1998). From this material lines with resistance to race F and with 34 chromosomes are being selected.

The results of this programme have demonstrated that the resistance found in perennial *Helianthus* species is suitable for breeding purposes since the transfer of resistance from twelve species with different ploidy levels has been achieved. The use of combinations of embryo culture and amphidiploid production is effective in

overcoming cross incompatibility and facilitates the transfer of resistance into diploid cultivated background. In most cases, the resistance found seems to be dominant, thus facilitating its transfer in backcross programmes.

The next step is to obtain derived lines breeding true for *O. cernua* resistance from the different species and to characterise the gene(s) controlling the resistance.

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PATHOGENIC VARIABILITY IN Orobanche cumana WALLR.

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Introduction.

Although two other *Orobanche* spp. (i.e. *O. aegyptiaca* Pers. and *O. ramosa* L.) are known to attack sunflower, *O. cumana* Wallr. is by far the most important parasitic angiosperm of this crop. *O. cumana* seems to be derived from the species *Orobanche cernua* Loefl., which was described attacking *Artemisia campestris* L. in central Spain as early as in the XVIII Century (Beck von Mannagetta, 1890). This species, considered autogamous, has been known for long to include two subspecies that differ in their host range. One (*O. cernua* ssp. *cernua*) attacks solanaceous plants (mainly tobacco, tomato and eggplant) whereas the other subspecies (*O. cernua* ssp. *cumana*) is specific of sunflower and *Compositae* (Parker and Riches, 1993). According to some researchers, this fact, along with some morphological and molecular differences between these two subspecies (Joel, 1988), justify the erection of the latter as the species *O. cumana* Wallr., which is closely related to O. *cernua* (Katzir *et al.*, 1996).

Broomrape of sunflower occurs mainly in south-eastern European countries such as Russia, Ukraine, Moldavia, Bulgaria, Romania and Yugoslavia, as well as Turkey, Israel and Spain. The importance of this parasitic weed to sunflower crops in Spain is relatively recent, particularly for oilseed cultivars, on which the first attacks were observed by the late seventies. By that time, confectionery sunflowers were severely attacked by broomrape, particularly in their traditional areas (González-Torres *et al.*, 1982). Severe attacks to oilseed hybrids became quite common since the early nineties, and hybrids resistant to all races known by then were released in Spain, providing a valuable control method. The resistance in these hybrids was broken down after a few years due to the appearance of a new race of the parasitic angiosperm.

Genetic variability of *Orobanche.*

Isoenzymatic studies with populations of *O. cumana* attacking either confectionery or oilseed type sunflowers from three different locations in southern Spain were carried out (Castejón-Muñoz *et al.*, 1991). A larger polymorphism was shown in the population that infected confectionery sunflower in the location with the longest history of broomrape infection. This suggested that only a selected subpopulation of the latter would be able to infect oilseed type varieties, and that relatively recent introductions of the parasite to new areas have also a restricted polymorphism.

PCR technique was shown useful to obtain reproducible and unique RAPD profiles, thus allowing the discrimination of different *Orobanche* spp. These profiles are not dependent on environmental and developmental factors as it is the case of isozyme markers (Katzir *et al.*, 1996). Furthermore, the same markers for *O. crenata* and *O. cernua* were valid to all populations tested, including several from Israel and some from Spain. Besides, genetic variation within *O. cumana* could be established after finding the appropriate RAPD markers. These genetic studies also suggest that *O. cumana* is most probably a self-pollinated species (Gagne *et al.*, 1998).

Resistance to broomrape and races of *O. cumana.*

Sunflower breeding, mainly for the increase in oil content, started in prerevolutionary Russia early this Century. Simultaneously, resistance to *O. cumana* was introduced in oilseed cultivars in the twenties, using *Helianthus tuberosus* L. as the most important source of resistance. Soon, populations of the parasite able to overcome resistance in these cultivars were found. Thus, races A and B of O. cumana were defined, resistance to race B was searched, and new cultivars were developed with resistance to both races (Skoric, 1988). More recently, new races of increased virulence appeared in eastern Europe as a response to selection pressure determined by the intensive cropping of resistant cultivars. Monogenic dominant inheritance was found in most cases, although there are some reports of more complex inheritance of resistance to *O. cumana* in sunflower (Domínguez, 1996; Ish-Shalom-Gordon *et al.*, 1993; Pogorletski and Geshele, 1976; Pustovoit, 1966; Saavedra *et al.*, 1994b; Sukno *et al.*, 1999; Vrânceanu *et al.*, 1986).

After the appearance of race C, also in the former USSR, a racial study in Romania concluded with the occurrence of five races (A-E). Sunflower differentials were established for these races and varieties resistant to them were developed. The

corresponding set of differentials (Kruglik A-41, Jdanov-8281, Record, S-1358, and P-1380) carried resistance genes Or_1-Or_5, respectively. Each of these genes has the peculiarity of providing resistance to all the previous races of *O. cumana* (Vrânceanu *et al.*, 1986). These five races of broomrape were also determined in Turkey and Bulgaria (Bulbul *et al.*, 1991; Shindrova, 1994). When the above-mentioned differentials were used to determine the broomrape races present in Spain, a variation from the original scheme was found. Broomrape populations from there attacked Record (resistant to races A-C, and susceptible to races D and E) but did not attack the previous differential in the set (Jdanov 8281) (Melero-Vara *et al.*, 1989).

Attempts to find molecular markers that discriminate DNA from populations of *O. cumana*, according to the race to which they belong, by means of RAPD analysis were made in Spain (Melero-Vara *et al.*, 1996). Polymorphic bands were obtained when some random primers were used, but there was no correlation between bands patterns and virulence of the parasite. In addition, genetic differences within some populations of broomrape were observed.

The occurrence of Spanish populations able to overcome resistance gene Or5 was shown, after several outbreaks since 1995, in fields of southern Spain cropped to sunflower hybrids resistant to races A-E of *O. cumana* (Alonso *et al.*, 1996). Following the racial designation from Romanian researchers (Vrânceanu *et al.*, 1986), these new populations overcoming genes Or_1-Or_5 should be considered to belong to race F (Melero, 1997). The same or a similar race was also found recently in Turkey.

During 1996-1998, several fields have been detected in southern Spain where this new race of *O. cumana* has been found. When molecular studies were made with one of them, a reduced intrapopulation genetic variability was found, as compared to the other broomrape populations tested. Evolutionary studies suggested two distinct groups of broomrape populations: one corresponding to those from eastern European countries, and the other to the ones collected in Spain, but probably the two groups have a monophyletic origin (Gagne *et al.*, 1998).

Eventually, markers for races of *O. cumana* will be found (Katzir *et al.*, 1996; Gagne *et al.*, 1998) but, until these become established, racial characterization must rely on the differential reactions of sunflower inoculated with populations of broomrape.

Races of *O. cumana* in Spain.

Several experiments of artificial inoculation of sunflower differentials were conducted in Córdoba, Spain to evaluate their reaction to 47 populations of broomrape attacking sunflower in different areas of Spain from 1989 to 1996.

The eight broomrape populations from 1989 showed a very low virulence (race A), except two of them that attacked one or three of the differentials with resistance genes Or_1-Or_3. Except for three populations of 1990 and 1991 with low virulence, the remaining 10 broomrape populations from these two years were virulent to Kruglik A-41 (gene Or_1) and Record (gene Or_3) but Jdanov-8281 (gene Or_2) and P-1380 (gene Or_5) remained fully resistant to all of them (Saavedra et al., 1994a). In contrast, only one out of the 14 populations of broomrape collected in 1992-1993 was unable to infect Kruglik A-41, but Record showed fully susceptible to all these populations; only one of them (CU 192) showed moderately virulent to differentials Jdanov-8281 and P-1380. Four out of nine populations of *O. cumana* collected from central Spain in 1994 were virulent to Jdanov-8281, whereas seven of them infected S-1358 (with gene Or_4), and all nine populations infected Record; five of these showed moderate or full virulence to differential P-1380 (Melero-Vara *et al.*, 1996).

The inoculation of nine of the most virulent populations collected from 1993-1996 on 10 sunflower lines indicated that all the nine populations attacked Kruglik A-41, and genes Or_2 and Or_5 were overcome by three of the populations tested (i.e. CU494, CU 996 and SE296). Only two sunflower lines, JD-6 and W-14, were fully resistant to all the broomrape populations, whereas two other (JM-1 and R-41) were resistant to all these populations except to SE296. Genes Or_2 and Or_5 were overcome by three of the populations tested (Sukno *et al.*, 1999).

In summary, the racial evolution of sunflower broomrape in Spain in the last ten years seems to have led to an increase in the frequency of populations B and F and a decrease for those of races A and E in Central Spain. In contrast, the practical substitution of race A by race B until 1994, was recently followed by a moderate decrease of this race, simultaneous to an increase in the frequency of populations belonging to races E and F in southern Spain (Melero-Vara, 1997).

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IMPACTS OF *Orobanche* ON HOST SOURCE-SINK RELATIONS

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Introduction.

The extent to which parasitic plants depend on heterotrophic sources of carbon differs markedly between species, ranging from complete dependency in holoparasites to complete autotrophy in those hemiparasites which are facultative, when unattached to host. The extent to which growth and reproductive output or yield of host plants is compromised by parasitic angiosperms varies enormously and, is not necessarily correlated with the degree of dependence of the parasite on its host. Furthermore, our understanding of the mechanisms by which parasites affect host vigour is incomplete and what we do know largely relates to parasites that are also agricultural weeds. Here, the impacts of *Orobanche* on host growth and carbon metabolism is discussed, and data presented to show that the interactions are largely source-sink driven.

Source-sink interactions between *O. cernua* and tobacco.

While there are many holoparasitic plants, those from the genus *Orobanche* have received the much research attention because of their importance as weeds of crops in Mediterranean regions. Tobacco infected with *O. cernua* increases the net flux of carbon from shoots to roots by 77%, and 73% of this carbon is removed by the parasite almost entirely through the phloem (>99%) (Hibberd *et al.* 1999). Further, *O. cernua* relies heavily on host phloem for inorganic solutes and it exerts a large impact on the nitrogen relations of the plant; nitrate uptake is stimulated and amino acid content of xylem sap is lower.

Since *Orobanche* spp. are such efficient sinks, we can examine the extent to which host responses are driven by source-sink interactions in these associations. In the tobacco-*O. cernua* association, for example, infected plants achieved only 29% of the biomass of control plants over a 73 day period (Fig. 1a). If dry weights of host and parasite are combined, however, biomass of the infected system does not dif-

fer significantly from that of uninfected tobacco plants (Fig. 1b), suggesting that the difference in biomass can be attributed solely to diversion of dry matter from host to parasite. This maintenance of productivity in the infected system, relative to uninfected tobacco, is achieved through a combination of physiological and morphological modifications in the host. First, the ratio of photosynthetic to non-photosynthetic tissue in infected plants is greater and second, leaf senescence of infected plants is delayed, resulting in a stimulation of canopy (by approximately 20%) and hence the supply of photosynthate (Hibberd *et al.* 1998, 1999).

Relative importance of sink demand and source strength.

The extent to which infection with *O. cernua* can stimulate the relative productivity of tobacco is limited, as illustrated by the observation that the number of *O. cernua* attached to host roots has little effect on the total dry weight of parasite tissue supported. Thus, as the number of attachments increases, the size of each *O. cernua* spikelet decreases (Fig. 1c, d). These data suggest that potential productivity of the host places an upper limit on biomass accumulation in the parasite, overriding parasite 'sink strength' as a determinant of host productivity (Hibberd *et al.*, 1998). This has been convincingly illustrated using Rubisco antisense technology to modify genetically productivity of tobacco plants and then infecting them with a single *O. cernua* parasite per host (J. M. Hibberd, W. P. Quick, J. D. Scholes & M. C. Press, unpublished). By decreasing Rubisco content, rates of photosynthesis (Fig. 2a) and carbohydrate supply to the parasite are also lowered. In this modified system there is a linear relationship between system biomass and maximum photosynthetic rate (Fig. 2b) and also between parasite biomass and host photosynthetic rate (Fig. 2c). Thus, as the productivity of the host is decreased, both host and parasite biomass decrease proportionally, as can be seen from the relative size of the *O. cernua* spikelet growing on hosts with different photosynthetic capacities (Fig. 2d). System biomass (host plus parasite) remains the same as that of uninfected tobacco with a similar photosynthetic capacity. Thus, *O. cernua* is able to modify productivity of its tobacco host up to a limit set by the source capacity of the host. This results in overall productivity of the infected system being comparable to that of uninfected tobacco.

Conclusions and other *Orobanche*-host associations.

Field-grown tobacco, tomato and faba beans infected with *O. ramosa* also have similar system biomass when compared with uninfected plants (ter Borg, 1986). In

contrast, productivity of tomato infected with *O. aegyptiaca* in a controlled environment study was sustained only when infection density was low or occurred early in the life cycle of the host. When parasite biomass increased beyond a critical level, system biomass was lower than that of uninfected control plants (Barker *et al.* 1996). Clearly, in this situation, the host plant could not compensate sufficiently (either by morphological or physiological means) to maintain the overall productivity of the system i.e. parasite sink strength dominated the interaction. In contrast, productivity of *Trifolium repens* infected with *O. minor* is enhanced by growth at elevated CO_2, but there is no difference in parasite biomass per host between plants grown at ambient or elevated CO_2 (Dale & Press 1998). Thus, in this association, sink strength of *T. repens* (and probably its *Rhizobium* symbionts) appears to be stronger than that of *O. minor*.

Ultimately, the balance between parasite sink strength and host productivity will be determined by host and parasite genotype, nutritional status of the host and environmental or experimental conditions.

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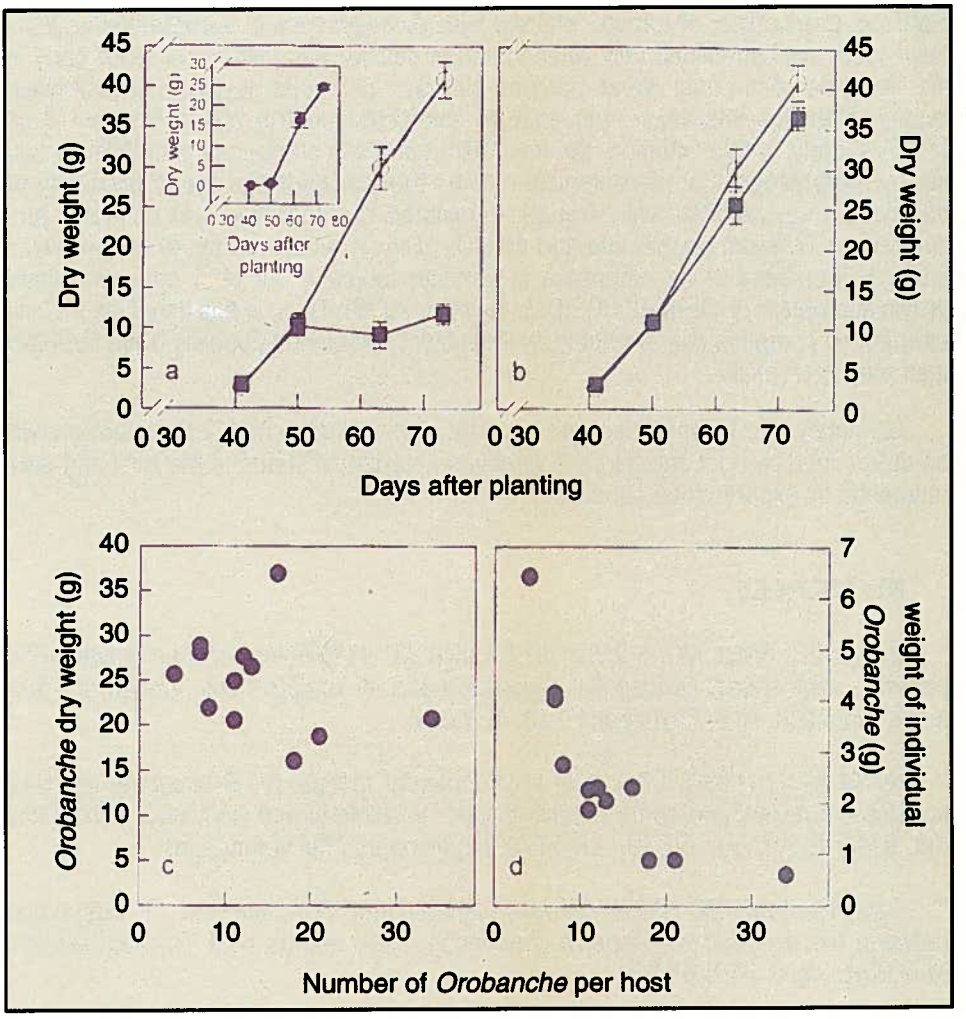

Figure 1. (a) The dry weight (g) of uninfected tobacco (○) or tobacco infected with *O. cernua* (■) from 41 to 73 days after planting. Inset is the dry weight (g) of *O. cernua* parasitising tobacco over the same time period. (b) The dry weight of uninfected tobacco plants (○) and the infected system (tobacco plus *O. cernua*) (■). All data are shown as means ± S.E. (c) The dry weight of *O. cernua* (g per host plant) as affected by the number of *O. cernua* attached to the roots of the host. (d) The dry weight of individual *O. cernua* spikelets (g) as affected by the number of *O. cernua* attached to the roots of the host.

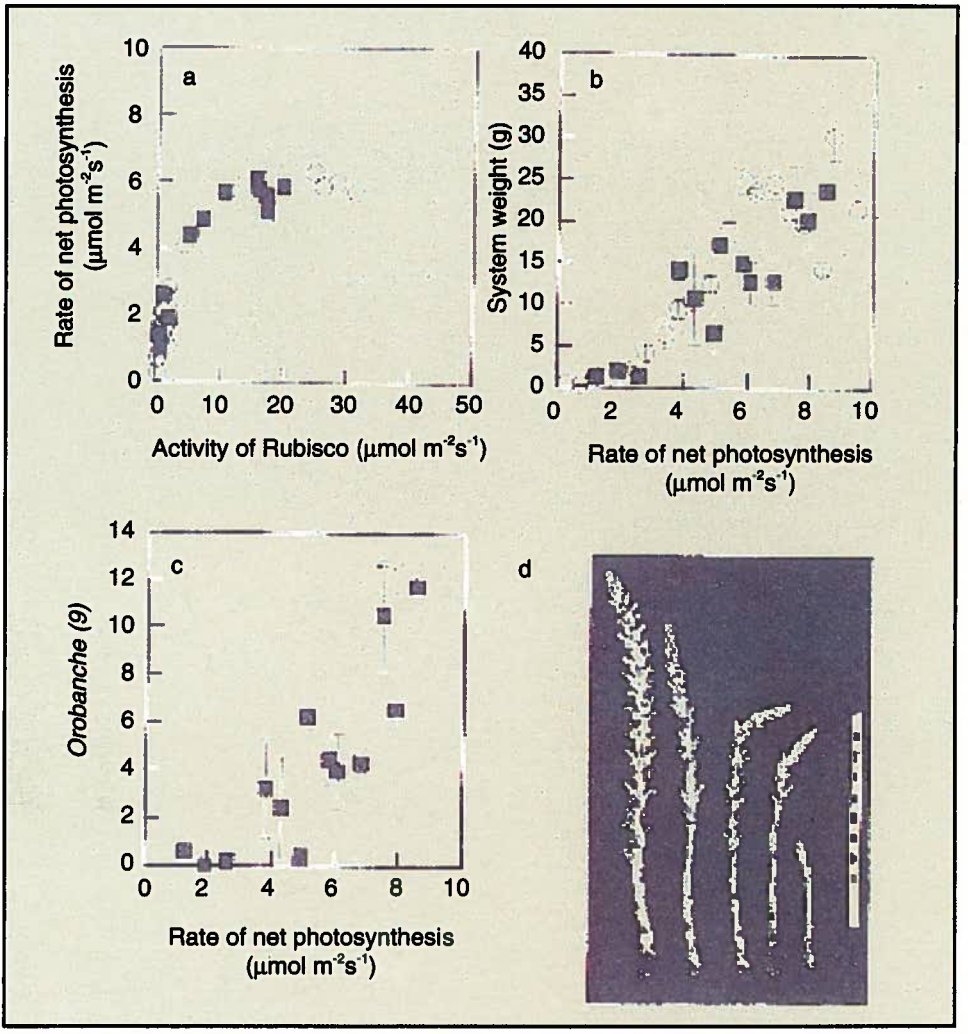

Figure 2. The relationship between; (a) the rate of net photosynthesis of uninfected tobacco plants (○) and plants infected with *O. cernua* (■) and the activity of Rubisco; (b) the total biomass of uninfected (○) and *O. cernua* - infected tobacco plants (■) and (c) the the biomass of *O. cernua* and the rate of host photosynthesis. (d) *O. cernua* spiklets from tobacco plants with, from left to right, decreasing rates of photosynthesis.

HOW PLANTS DEFEND THEMSELVES AGAINST ROOT PARASITIC ANGIOSPERMS: MOLECULAR STUDIES WITH Orobanche spp.

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SUMMARY

On the basis of the results obtained by our research group as refers to the sunflower (*Helianthus annuus* L.)-broomrape (*Orobanche* cernua Loefl./*Orobanche cumana* Wallr.) interaction and others found in the literature involving *Orobanche* spp., we will present different hypotheses, from a molecular perspective, for explaining how crop plants can defend themselves against broomrape parasitism. References to *Striga*, as it is the best known parasitic weed, are unavoidable and when illustrative, are also included. Resistance to parasitic weeds can be a complex multifactorial component, each component being more or less important depending on the host (species, varieties and populations), the parasite (species and races or populations), and environmental factors (both biotic and abiotic). A priori, and considering the complex life cycle of the parasite, which is very well known at the citological level but not at the molecular one, different host plant strategies can be proposed. Each strategy is directed at interrumpting the development cycle of the parasite at each step, the sooner the better, in terms of efficacy: germination, haustorial induction, attachment, penetration and installation, development, emergence and flowering. Once known the molecular bases which govern the plant-parasitic angiosperm interaction, different strategies to prevent parasitism can be proposed, included design of agrochemicals and the obtaining of high resistant varieties through plant breeding or genetic engineering techniques.

INTRODUCTION

There are in nature an ample number of cases of angiosperm-angiosperm symbiotic interactions in which one of the components (parasitic weed) needs, in order to complete its biological cycle, parasite other plants (host), this interaction being very specific. The number of parasitic angiosperm species so far described are about 3000, belonging to approximately 16 different plant families (Press and Graves, 1995). However, a specific species is only able to parasite a reduced number of host plants (A. Pujadas, this volume). According to this, the obvious question is: which factors determine the susceptible or resistant character of a species (non-host resistance) of a variety whithin a species (host-resistance; also considering tolerance), and hence, the establishment of a compatible or incompatible interaction? Three factors can be considered in order to answer this question; first, the host, second, the parasite and third, the biotic and abiotic enviroment. In this review we will exclusively focus on the host, other chapters of this volume will deal both with the parasite and the environment. Our approach is biochemical or molecular, and consequently, we do not go into genetic aspects of the interaction, that is how resistance inherits and whether it is a monogenic (qualitative) or multigenic (quantitative) character; these aspects have been covered in other chapters of this monograph. Additionally, and in order to have a clear idea about resistance, other aspects must be clarified, like the alogamous or autogamous character of *Orobanche* spp., and a clear, simple and rapid technique which allows the characterization of the different populations (races) of the parasite, since resistance mechanisms, as indicate above may operate only against specific races or populations of the parasite.

HYPOTHESES

Since we started in parasitic plant research, in 1995, in collaboration with Prof. Luis García-Torres (IAS, CSIC, Spain) two hypotheses directed our investigations. The first was to admit the existence of different host-plant strategies to prevent root parasitism, each strategy directed at specific stages of the biological cycle of the parasite. According to this we decided to optimize bioassays for studying each stage (Jorrìn *et al.*, 1995). Today, and ample number of bioassays are available for that purpose, some of them including *in vitro* plant tissue culture techniques. However, care must be taken when extrapolating laboratory results to the field, as the climatic, soil, biotic surrounding and other external factors can modify the resistant or susceptible phenotype. The second hypothesis, while considering similarities between the infection process of a fungi and a parasitic weed, was to admit that, at least at the very

early stages, the same defence mechanisms operating against fungi can work against parasitic weeds. In this respect there is to much to be learnt from 25 years experience in molecular plant pathology (as an example, readers are referred to Jackson and Taylor, 1996; and the monographic volume on plant-microbe interactions corresponding to vol. 1 (4) of Current Opinion in Plant Biology, August 1998). Also, the inclusion of experimental model systems which, like tobacco and *Arabidopsis* (Yoder, 1997; Westwood and Foy, 1998), are very well known at the molecular genetic level and for which there are an ample number of transgenic plants and mutants available would help us to find out more in detail about plant-parasitic plant interaction.

Both of the above mentioned hypotheses fit in very well with what is known about resistance to *Orobanche*. Resistance is mainly established during the germination and installation process. In the sunflower-*O. cumana* interaction we have observed that in a resistant variety there is no tubercle formation (Jorrin *et al.*, 1996), this observation being extended to other resistant sunflower commertial hybrids or wild *Helianthus* spp. (Ruso, 1997; Thalouarn *et al.*, 1998), and host-crops resistant to other *Orobanche* spp. (covert in this volume), although the mechanism differs for individual specific interactions.

Tolerance to *Orobanche*, as is the case of confectionery sunflower varieties, understood as the host plant supporting broomrape growth to mature flowering and seed production with no important reduction in host plant growth, development and yield, also deserves attention and, despite the minimum interest devoted to it, could be a solution for the parasitic plant problem. Tolerance depends on physiological factors associated with plant growth regulators, hydric regime and source-sinks relationships, this being covered by M. Press.

The following possible resistance mechanisms are discussed, appearing separately and referring to the stages of the biological cycle of the parasite they affect: conditioning, germination, haustorial induction, attachment, penetration and installation, development, emergence and flowering. Neither conditioning, as it takes place in the absence of a host, haustorium induction, as it has not been studied in *Orobanche*, nor flowering and seed production, as it takes place in susceptible or tolerant hosts, will be considered.

MECHANISMS OF RESISTANCE

1. Germination

The germination of root parasitic plant seeds depends on chemicals exuded from the roots of the host plant. The chemical nature of such germination stimulants is well known in the case of *Striga* (Butler, 1995). Little is known with respect to *Orobanche* germination stimulants. Only in a recent report (Yokota *et al.*, 1998), two germination stimulants for *Orobanche minor*, alectrol and orobanchol, have been isolated from the root exudate of its host *Trifolium pratense*. We also have evidences (Perez de Luque *et al.*, 1999) indicating that certain sesquiterpene model molecules related to strigol and to those described in sunflower induce *O. cumana* seed germination but not that of other *Orobanche* spp. tested (*ramosa, aegyptiaca* and *crenata*). A number of plant growth regulators have been proven to induce parasitic seed germination *in vitro* (Wegmann, 1996), and a recent report indicates that jasmonate, a signal molecule involved in plant resistance to diseases, promotes *Orobanche* seed germination (Yoneyama *et al.*, 1998a). The role of plant grow regulators in inducing germination must be explained *in vivo*. Others compounds, like some agrochemicals (Logan and Stewart, 1992; Wegmann, 1996) also induce germination, although their effect could be non-specific or they act by inducing ethylene biosynthesis (Logan and Stewart, 1991).

A lack of production of germination stimulants could be the clearest mechanism for horizontal or non-host resistance, with the exception of trap crops. In interactions involving *Orobanche*, there are cases of both host resistant and susceptible varieties inducing seed germination. However, as we have observed, sunflower resistant varieties induce *O. cumana* seed germination at a much lower rate than the susceptible one (Jorrin *et al.*, 1998), although there are cases in which such a correlation has not been found (Thalouarn *et al.*, 1998) and even cases in which resistant varieties induce germination at higher rates than the susceptible one (Goldwasser *et al.*, 1997).

Once the chemical nature of the germination stimulant is known, it opens up various possibilities for crop protection strategies, including: i) the design of agrochemicals which cause suicidal germination; ii) the selection of plants (crop plants) or microorganisms which excrete germination stimulants, thus bringing about suicidal germination. There are some reports indicating the production of germination stimulants by microorganisms (Visser, 1975; Yoneyama *et al.*, 1998a,b) and this will give opportunities to the so-called 'biological control'; iii) the obtaining of new crop varieties, through plant breeding, which produce low amounts of germination stimu-

lants or through genetic engineering directed at blocking the synthesis and/or excretion of the germination stimulants by, for example, antisense techniques.

Besides the production and excretion of germination stimulants, host plants can also produce and excrete toxic compounds for the parasite which either inhibit the germination or cause necroses in germinated seeds. This hypothesis, first indicated by Whitney (1978), has been firmly supported in the sunflower-*O. cumana* system (Al-Menoufi *et al.*, 1996; Jorrin *et al.*, 1998, and unpublished results). However, not too much attention has been paid to this phenomenon. From a host plant perspective, it seems logical to establish a first defensive line (a war away from home) aimed at preventing parasitic weed seed germination or the killing of germinated seeds. This strategy, based on the excretion of allelochemicals, is also used against competitive neighbouring plant species. The importance of this phenomenon can be understood by taking into account that as much as 20% of a plant's net photosynthesis is released into the rizosphere and that approximately 120 Kg/ha plant-derived phenolics can be added into grassland soil annually (Estabrook and Yoder, 1998); many of these strongly affect neighbouring plant and microbial communities (Siqueira *et al.*, 1991; Baker *et al.*, 1997). Sunflower resistant varieties excrete higher amount of coumarins (scopoletin and ayapin) than the susceptible one in response to *O. cumana*. Both compounds inhibit the broomrape seed germination induced by GR-24 and cause necrosis of the germinated seeds (Jorrin *et al.*, 1998). Curiously, a paper by Worsham and Klingman (1962) indicated that scopoletin induced Striga germination. We are currently studying if coumarin production and excretion is a resistance factor for the different interactions (including broomrape races and commercial sunflower hybrids and wild relatives) and if their synthesis and excretion can be controlled by agrochemicals as a strategy for *Orobanche* control in sunflower.

2. Attachment

Before penetrating, the parasite attaches to the host root surface (Press and Graves, 1995; Joel *et al.*, 1996). The production of mucilaginous adhesive secretions, probably in response to chemical or mechanical host signals, has been proved in the case of *Orobanche* (Joel and Losner-Goshen, 1994), although other mechanisms may operate, including the simplest support given by soil compactation (Press and Graves, 1995). The contact between host and parasite in root inner tissues mediated by secreted substances can take place in cases in which haustorial cells fail to penetrate host tissue, as for example in the presence of non-host or resistant-hosts (Joel *et al.*, 1996). The fact that attachment in some species takes place to

many biological inert or plastic supports, seems to indicate that it is fairly non-specific. However as it is known that not all the haustoria make functional attachments and that attachment frequencies are lower and non-uniform on resistant root-surfaces (Press and Graves, 1995), there is a possible existence of resistance mechanisms impeding or making attachments difficult and, hence, possible infection sites, reasons for studying this stage and trying to exploit it.

3. Penetration and connection to the vascular system

Following attachment and before connecting the vascular system, root parasites must pass the epidermis, cortex, endodermis and central cylinder. Then, the haustorium intimately connects the phloem and xylem vessels and both partners' sieve elements share plasmodesmatal counections (Dorr, 1996). According to the available data, *Orobanche* host resistance is stablished during this stage (Lane *et al.*, 1997; Thalouarn *et al.*, 1998; Jorrìn et al., 1996, 1998 and unpublished results).

The penetration process by *Orobancke* has been investigated and reviewed by Dr. Joel. The parasite uses mechanical forces as well as extracellular hydrolitic enzymes as mechanisms for host-tissue penetration (D. Joel, this volume). Although not documented, host plant inhibitors of the parasite enzymes which like pectinases facilitate host root penetration can play a role in preventing tissue invasion by the parasite.

A number of defence reactions, both constitutive and inducible, as has been firmly documented in the case of phytopathogens could be useful in preventing parasite invasion (Jackson and Taylor, 1996), including both preformed passive mechanical or chemical barriers, providing non-specific protection against a wide range of species, as well as inducible active host-specific responses, providing varietal specific responses, despite the fact that these can also be manifested in non-host species (Hood *et al.*, 1998). Following we will deal with inducible active host-specific responses.

Hypersensitive response (hypersensitive cell death, HR) is very well documented as a defence reaction against pathogenic microorganisms (virus, bacteria and fungi) and the triggering of such phenomenon is very well documented at the molecular level (Heath, 1998). HR may either block the radicle penetration through the host root, the development of the haustorium and the connection to the host vascular tissue. In the sunflower-*O. cumana* experimental system, we have observed both host-root necrosis and necrosis of the broomrape radicle. There are also a number of cases in which hypersensitive necrosis in resistant-host and non-host plants take

place around sites of penetration for *Orobanche, Striga* or *Cuscuta* (Dorr et *al.*, 1994; Lane *et al.*, 1997; Goldwasser et al., 1998). Hypersensitive cell necrosis is accompanied by the accumulation of a number of toxic compounds, among them phytoalexins, active oxygen especies (AOE) and the so called pathogenesis-related proteins (PRPs). Although it would not be surprising, as far as we know, there are no published data on the production of AOE in response to parasitic plants.

It was Dr. K. Wegmann who first proposed the hypothesis of the role of phytoalexins as defensive compounds against parasitic weeds (Wegmann *et al.*, 1991). In the last 4 years we have investigated the possible defensive role against *O. cumana* of a family of sunflower phenolic compounds, the simple 7-hydroxylated coumarins scopoletin and ayapin. Scopoletin and ayapin are multidefence secondary metabolites produced by sunflower, their biosynthesis being induced in response to biotic and abiotic stresses and to different chemicals and agrochemicals (Gutiérrez-Mellado, 1998; Serghini, 1999). These compounds have been reported as phytoalexins, insect-feeding deterrents and allelochemicals (Jorrìn, 1999). In addition, they can also have an important defensive part against broomrape parasitism by preventing the germination (allelopathic effect) and successful installation (phytoalexin effect) of the parasite. This hypothesis is supported by the following experimental data: i) both compounds inhibited the *in vitro O. cumana* seed germination induced by the strigol analogue GR-24 and induced necrosis in germinated seeds (Jorrin *et al.*, 1996; Pérez de Luque, 1998; and unpublished results); ii) a higher accumulation of both coumarins in root tissue and higher root-excretion was observed in resistant sunflower varieties in response to broomrape infection (Jorrin *et al.*, 1998; Serghini, 1999). In tobacco, parasitization by *Orobanche* induces expression of hmg^2, a gene coding for a hydroxy-3-methylglutaryl CoA reductase, the rate limiting enzyme in the synthesis of sesquiterpenoid phytoalexins; its induction takes place at the infection site and requires host root penetration being continuous and associated with secondary (Westwood *et al.*, 1998).

The involvement of pathogenesis-related proteins (Kombrink and Somssich, 1997) in the defence against parasitic plants has only been, as far as we know, reported in tobacco. Transgenic tobacco plants transformed with a PRb-1b-GUS chimeric gene show higher GUS activity when infected by *Orobanche*, and the expression limited to the infection site (Joel and Portnoy, 1998).

Increased lignification of the host cells around the penetrating radicle, or of the host xylem elements, has been reported for sunflower resistant varieties to *O. cumana* and other plants (both host and non-host) resistant to different *Orobanche* spp.

(Antonova, 1994; Shomer-Ilan, 1993; Dorr *et al.*, 1994; de Ruck *et al.*, 1995; Mayer *et al.*, 1997; Goldwasser *et al.*, 1998), in some cases accompanied by an accumulation of phenolic compounds (Ish-Shalom-Gordon *et al.*, 1994) and related enzymes, like PAL, laccases and peroxidases (Goldwasser *et al.*, 1998; Serghini, 1999). However, this reaction not always prevent rapid and successful penetration by the parasite (Mayer *et al.*, 1997). This could be the case, for example, if the penetration of the germ tube occurs along the middle lamellae, as it has been reported by Joel and Losner-Goshen (1994); in this case, only when the vascular cylinder is reached, the lignin content can be a stopping for the invasion.

In Dr. Cubero *et al.*'s review (1994) there were clear statements indicating the direction to be taken in parasitic plants research: '... Although some resistant cultivars have been identified in several crops, great gaps exist in our knowledge of the parasites and the genetic basis of the resistance... Molecular techniques have yet to be used to locate resistance to parasitic angiosperms. While intensifying the search for genes that control resistance to specific parasitic angiosperms, the best strategy to screen for resistance is to improve the already existing *in vitro* or greenhouse screening techniques'. Five years later, we are in a position to supply plant breeders with molecular data to conduct their programmes for obtaining resistant varieties. Also, as is the case of plant resistance to microorganisms, new varieties more resistant to parasitic angiosperms obtained by genetic engineering techniques can be hypothesized (Dong, 1998), although some of them be unsuccessful, i.e. transgenic tobacco overexpressing hmg^2 and showing increased resistant to viral and bacterial pathogens were as equally susceptible than control tobacco plants (Westwood *et al.*, 1998). Finally, it is necessary to try to exploit the phenomenon of induced resistance (Ryals *et al.*, 1996) by chemical or biological treatments as these have proven to be efficient for controlling pathogens; some of us have started to do experiments in this direction.

4. Development

Once the parasite has successfully contacted the vascular tissue of the host it starts to deliver water and nutrients and other necessary compounds, and hence, to compete with host plant developing organs. During this stage which includes the formation of tubercles and its ulterior growth and stem development it is possible that the host plant activates defence reactions. In cowpea, a different mechanism of resistance to S. *gesnerioides* is observed in variety B301, in which parasitic growth is severely restricted, with tubercles remaining less than lmm in diameter, with a limi-

ted stem development. This growth reduction has been explained on the bases of the reduced vascular connections between host and parasite or by an inadequate supply of plant regulators (Lane *et al.*, 1997). Alternatively, it is also possible that the host plant delivers to the parasite, either via xylem or phloem, toxic compounds or inhibitors which negatively affect tubercle growth and development. In *Orobanche minor* Smith parasiting *Trifolium repens* L. (clover) endogenous and host delivered gibberelins have been detected (Suzuki *et al.*, 1994). Factors controlling the developmental timing of a putative host, source sink relationships, as well as the hydric regime (stomatal conductance, transpiration rate, water potential, osmotic pressure), can determine the susceptible, resistant or tolerant character of a plant (Wegmann *et al.*, 1991; Press and Graves, 1995; Press, this volume).

CONCLUSIONS

We have just started to understand the molecular basis which govern the plant-parasitic plant interaction, although most of the knowledge has been generated with *Striga*, being also neccessary to direct our effort to other parasitic angiosperms which like *Orobanche* are causing yield losses in important crops. Agressiviness as well as resistance factors are being characterized at the molecular and genetic level, and in accordance, different control strategies can be evaluated, including the design of agrochemicals which cause suicidal germination or trigger induced resistance, crop rotations by using new trap crops, biological control by using microorganisms and selecting new more resistant varieties by traditional plant breeding or genetic engineering techniques. By now quantitative, multigenic resistance can be understood but, how far are we from characterizing qualitative, monogenic, resistance?. An open question, following the experience with sunflower, is the durability of the resistance developed by plant breeding programmes. Early this century wheat rust resistance was shown to segregate as a dominant Mendelian trait and in the 1940's Flor proposed the gene-for-gene hypothesis, but was not until the 1990's that both avirulence and resistance genes started to be characterized. Now we have a powerful methodology and extensive molecular phytopathology background. These together with the use of experimental model systems like *Arabidopsis* and tobacco, will help us to progress in this area.

ACKNOWLEDGEMENT

Thank you very much to Dr. L. García-Torres who gave us the opportunity of starting into the parasitic plant field. Our research has been supported by the Spanish CICYT (AGF 95-0103 and 97-1230-CO2) and the Consejería de Educación y Ciencia de la Junta de Andalucía (AGR 0164)

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POTENTIAL OF *Phytomyza orobanchia* FOR THE BIOLOGICAL CONTROL OF *Orobanche* spp. AND ITS POSSIBLE APPLICATION

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Introduction.

Parasitic plants of the genus *Orobanche* cause serious damage in several crops. In Morocco, *Orobanche crenata* Forsk. presents a serious problem for food legumes, especially faba bean (*Vicia faba* L.), but also for pea (*Pisum sativum* L.), lentil (*Lens culinaris* Medik.) and currently for pigeon pea (*Cicer arietinum* L.) (Bouhatous, 1987; Lutzeyer *et al.*, 1994).

The chemical control of *Orobanche* with herbicides is difficult. This is because the chemicals must enter the weed via the vascular system of the host, in order to exert an effect on the parasite. Thus, the latter may also be susceptible to damage. The relative efficiency of chemical control is restricted for the moment only to faba bean. Control methods, like the use of herbicides and the breeding of *Orobanche* - resistant varieties, have to be adapted to each individual crop and *Orobanche* species. On the other hand, biological control has the advantage of being applicable to all host plants and to be effective against all species of *Orobanche*. The agromyzid fly *Phytomyza orobanchia* Kalt. is particularly suitable for biological control, due to its marked selectivity and to its high efficiency (Kroschel *et al.*, 1996). The "inundative approach" is based on an increase in the population of the antagonist by mass release. The manipulation of the density of the antagonist population, by reducing direct mortality inflicted by cultural practices and secondary enemies, leads to a synchronisation between the development of the parasitic plant *Orobanche* and the antagonist *P. orobanchia,* and consequently to an increase of its efficiency. The bio-

logical control of *Orobanche* with *P. orobanchia* interferes with the sensitive reproduction stage of the biological cycle of *Orobanche*. The impact of the biological control agent is the reduction of seed production and, as a consequence, a lower input to the soil seed bank. The depression of seed production by *Orobanche* prevents the supplementary infestation and dissemination. Although *P. orobanchia* can severely reduce the seed production, the longevity of seeds in the soil seed bank (10 – 15 years) results in the potential infection of crops in the subsequent growing seasons. Therefore efficient control demands continuous application over several years.

Basic information on the biology and the population dynamics of P. orobanchia as well as on interactions of the different trophic levels under the geographical and climatic conditions of Morocco are studied (Klein, 1995; Kroschel *et al.*, 1996). The central elements of this study were the dynamics of the infestation of *O. crenata* by *P. orobanchia* in the long term and the impact of mortality factors as parasitism. In particular, the efficiency of inundative releases of *P. orobanchia* in the field in the short and long term and possibilities of its application were investigated and evaluated.

Material and methods.

Observations concerning the impact and limiting factors of *P. orobanchia* under natural conditions have been undertaken during the years 1994, 1996, 1997 and 1998 in the Saïss region in the central North of Morocco. The reduction of the seed production by the infestation of capsules by P. orobanchia is calculated on the basis of the comparison of the number of seeds of 72 not - infested capsules with that of 72 infested capsules. The counting of seeds has been made according to the method described by Hammad *et al.* (1967). The viability of seeds is determined according to the T.T.C. method described by Linke (1987). The efficiency of inundative releases of *P. orobanchia* was tested by field trials during the three agricultural seasons 1996, 1997 and 1998 with cages allowing controlled releases installed at the Experimental Station in Douyet on a plot heavily infested by *O. crenata*. The design of the trial was a random block with plots of 3.5 m x 4 m with 3 different treatments and 4 repetitions each. The inundative releases were evaluated in cages covered by a fine gauze installed in a faba bean field as host plant for *O. crenata*. Open air plots served as controls without inundative releases but containing the natural population density of *P. orobanchia*. Based on different experiences and the biological data of *P. orobanchia* in Morocco, a formula for the calculation of the number of flies to release was established. The number of flies to be released may be calculated by multiplying the number of expected seed capsules / ha by the following factor: 2.1172 x 10-3. For the

evaluation of different storage methods, each samples of 1 kg of *O. crenata* shoots were stored in soil, in cloth bags, in a "Phytomyzarium" and in a refrigerator.

Results.

The rate of naturally infested seed capsules was 49.0, 44.5, 55.4 and 51.4% in 1994, 1996, 1997 and 1998, respectively. Variations in the rate of infested capsules under natural conditions depended essentially on climatic factors. The drought in 1993 and 1995 resulted in the absence of the host plant faba bean, and therefore also of *Orobanche*. Consequently, the density of the population of *P. orobanchia* remained weak, especially in the following year since the density of the initial population is responsible for an efficient infestation of *Orobanche* seed capsules. Raised average temperatures in January, February and March 1997 and 1998 caused an early emergence of *P. orobanchia* by three weeks and *O. crenata* by two weeks in comparison to 1994 and 1996. The first and second generation of P. orobanchia thus developed earlier, resulting in a higher infestation rate 1997 and 1998. In other countries infestation rates of *O. crenata* parasitizing faba bean range from 32.5 to 94 %. The highest infestation rate was reported from Turkey (94 %) (Nemli and Giray, 1983), the lowest from Syria (32.5 %) (Linke *et al.*, 1990). In Egypt, infestation rates between 82 and 89.4 % were observed (Hammad *et al.*, 1967; Tawfik *et al.*, 1976).

95.5 % of seeds in a capsule are destroyed when infestation with *P. orobanchia* occurs. Equivalent data were found in Egypt (89 %) and Syria (91.1 %) (Hammad *et al.*, 1967; Linke *et al.*, 1990).

P. orobanchia was parasitized by 9 hymenopterous species of the families *Eulophidae* (*Pronotalia orobanchiae* (Graham) and *Baryscapus phytomyzae* (Kostjukov)), *Pteromalidae* (*Sphegigaster* sp. (near *cuscutae,* Ferrière) and *Callitula bicolor* (Spinola)), *Braconidae* (no. 406, not determined) and no. 800, 1000, 1200 and 1300 (not determined). Total parasitization rates were 6.4, 4.0, 11.4 and 7.8 % in 1994, 1996, 1997 and 1998, respectively. The most abundant parasitoid was *Pronotalia orobanchiae*. In general observed parasitization rates were quite low compared to parasitization rates reported from other countries. In Bulgaria and Moldavia maximum parasitization rates of 81.7 and 92 %, respectively, were observed.

Trials of inundative releases of *P. orobanchia* in cages in the field have shown that the efficiency of *P. orobanchia* can be increased significantly. Only 3.7% (1996) to 6.2% (1997, 1998) of seeds were viable in comparison with 94.9 (1996), 34.1

(1997) and 36.5 % (1998) without inundative releases. The destruction of seeds is caused directly by the mining activity of *P. orobanchia* larvae, which devour the seeds in the capsules. Further larvae mining in shoot tissues cause indirectly the degeneration of capsules. Despite the low rate of seeds produced in the trial plots of inundative releases, the seed bank has increased in the short term. Although only 4 to 6% of viable seeds were able to develop in plots with inundative releases, the seed bank of *Orobanche* in the soil has multiplied by 6. This phenomena can be explained if one takes in consideration the situation of the *Orobanche* infestation at the Experimental Station in Douyet and, consequently, the large number of seeds which 3.7 % of the *Orobanche* seeds represent. With an average infestation of 206 *Orobanche* shoots per m^2 in Douyet in 1996, 4000 capsules each with 1700 seeds developed. Thus, a viability of 3.7% still represents an input of 251600 seeds per m^2 to the soil seed bank. It remains to be verified whether the efficiency achieved with inundative releases is sufficient to reduce the seed bank of *Orobanche* in strongly infested fields as at the Experimental Station in Douyet in the long term. Normally faba bean is cultivated only every two or even three years in rotation with cereals. During this period the seed stock is reduced by natural seed decomposition in the soil. In fields with a weak average infestation, the number of flies to be released may be lower and it can be supposed that the efficiency of inundative releases, the high level of seed destruction, has an effect on the seed bank.

The number of flies to be released calculated by the proposed formula may appear to be quite high. Thus the question is whether the "mass-rearing" method is efficient enough in order to obtain the necessary number of flies. The hatching rate of pupae of *P. orobanchia* is very low. In the case of the "rearing" method by storage in a Phytomyzarium, only 4.0 % of the pupae hatch the subsequent season. In years with a normal development of the host plant as well as of *Orobanche*, the number of hatched flies is more than sufficient even for highly infested fields (Tab. 1). In years with a poorer development of *Orobanche* and its antagonist *P. orobanchia*, the number of hatched flies can be too small for efficient releases. A minimum of 1.6 pupae per shoot is necessary in order to obtain enough adult *P. orobanchia* for release. In order to increase the very low number of hatching pupae, different storage methods were tested. The storage of *Orobanche* with *P. orobanchia* in a refrigerator (10.5 %) gave the best results. The low hatching rate (0.3%) observed on storage in soil at a depth of 10 cm is inadequate to allow use of this method.

Additionally, different artificial diets have been developed and tested but were not successful. Further research is necessary to develop efficient, simple and cheap mass rearing methods.

Conclusion.

The evaluation of the efficacy of inundative releases in Morocco has shown that the infestation rate of *Orobanche* by *P. orobanchia* could be increased considerably, and thus the production of viable *Orobanche* seeds could be reduced to only 3.7 to 6.2%.

The available "mass rearing" methods (storage in a *Phytomyzarium* or cloth bags) are not very efficient. High quantities of *Orobanche* shoots have to be collected and stored to get sufficient *P. orobanchia* for the field release. Improved storage methods as well as artificial diets for mass rearing of *P. orobanchia* are necessary to facilitate the application of this inundative approach.

It remains to be verified whether the observed reduction in the seed production of *Orobanche* is sufficient to reduce the seed bank in the long term. In the case of heavily *Orobanche* infested areas the biological control approach with *P. orobanchia* is not sufficient as a single control method. However, it could be used as an part of an integrated control approach in combination with other control methods as resistant cultivars or herbicide application. In such a combination, biological control can result in an effective reduction of the seed bank, which is one of the main components of integrated control approaches. In addition, in weakly infested areas, this approach could help to prevent further dissemination and infestation immediately.

Recommendations.

Further research work is necessary to develop an efficient biological control approach of *Orobanche* by *P. orobanchia*. The efficacy of inundative release of *P. orobanchia* observed in field trials with cages should be verified by field trials, involving the release of the antagonists in the open field, without cages. Emphasis should be placed on the development of techniques to enhance the efficiency of the antagonist by reducing direct mortality factors (cultural practices and natural ennemies). More efficient "storage" methods and, in particular, mass production techniques using artificial diets for mass rearing of *P. orobanchia* should be developed.

Key words.

Orobanche spp., *Phytomyza orobanchia*, biological control, inundative approach, mass rearing, mass releases, Morocco

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Tab. 1: Flies to be released and hatched flies stored in a Phytomyzarium per ha at different *Orobanche* intensities

Orobanche shoots/m²	flies to be released	pupae/shoot (1994)		hatched flies/ha shoots	
		1st year	2nd year	1st year	2nd year
0.1	65	5.4	7.9	215	316
1	650	3.5	5.1	1 380	2 029
10	6 500	3.2	4.7	12 917	18 988
100	65 000	2.0	3.0	81 429	119 702

SPECIES OF THE FAMILY *Orobanchaceae* PARASITIC OF CULTIVATED PLANTS AND ITS RELATIVES GROWING ON WILD PLANTS, IN THE SOUTH OF THE IBERIAN PENINSULA

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Introduction.

The aim of our present work is to determine accurately the broomrapes, not only those parasiting on cultivated plants but also its relatives growing on wild plants. Orobanche species are very well known to present taxonomic problems and also difficulty of its determination. The correct identification of any weed plant is a very important target for the weed researcher, because to find a method of control of the weeds, the first step is to know accurately what species we are dealing with.

Main Orobanche species.

The species of the genus *Orobanche*, in Europe, can be grouped in two Sections that can be clearly separate by the following characters:

1) Sect. TRIONYCHON Wallr.: Stems simple or branched; flowers with a bract and two bracteoles adnate to calyx; calyx with cylindrical to campanulate tube and four subequal teeth; corolla blue or violet, rarely white or cream.

2) Sect. OROBANCHE L.: Stems simple; flowers with a bract but without bracteoles; calyx generally divided into two lateral segments, segments entire, equally or unequally bifid or bidentate; corolla white, yellow, brown or red.

1.1. In the Section Trionychon the main parasite of agricultural plants is *O. ramosa* L. subsp. ramosa that nowadays grows (in our territory) on *Nicotiana tabacum* and in the past was common in traditional crops like *Cannabis sativa* (hemp) or *Linum usitatissimum* (flax). This taxon always parasites cultivated plants, and can be charac-

terised by its stem to 31 cm, branched, calyx 4-6 mm and corolla 12-15 mm, whitish or pale blue at apex. *O. ramosa* has often been confused with *O. mutelii* F.W. Schultz [=*O. ramosa* subsp. mutelii (F.W. Schultz) Coutinho] or with *O. nana* (Reuter) G. Beck [=*O. ramosa* subsp. nana (Reuter) Coutinho]

O. mutelii presents the stem to 17 cm simple or branched, calyx 7-10 mm, corolla (15)17-20 mm, pale to bright blue and always parasite on wild plants. Instead *O. nana*, very close to *O. ramosa*, characterised by its stem to 12 cm, calyx 5-8 mm and corolla 12-17 mm bright blue, found parasite on the cultivated *Pennisetum clandestinum* (kikuyu grass) but usually on wild plants *[Brachypodium ramosum, Polypogon monspeliensis (Poaceae), Leontodon taraxacoides, Calendula arvensis, Urospermum picrioides, Lactuca tenerrima, Asteriscus maritimus, Hedypnois cretica, Coleostephus myconis, Sonchus oleraceus (Compositae)]*

1.2. The other very well known broomrape, from this section, parasiting on agricultural plants, is *O. aegyptiaca* Pers. that grows on *Cucumis sativus, Gossypium herbaceum, Solanum melongena, Nicotiana tabaccum* among others plants. It is a specie from Centre and South West Asia, Middle East and South East Europe, not found in the Iberian Peninsula, but a very close taxon, *O. tunetana* G. Beck, has been found in Alicante (SE of the Iberian Peninsula) (Pujadas *et al.*, 1997).

O. tunetana was only known from North Africa and firstly identified as *Phelipaea aegyptiaca* sensu Kralik and has also been named as *O. aegyptiaca* subsp. tunetana (G. Beck) Maire. It differs, from *O. aegyptiaca*, in its smaller leaves 0.5-1.0 cm (vs. o.5-1.5 cm in *O. aegyptiaca*), upper leaves lanate glandular (vs. hairy glandular), smaller corollas (14)16-20(23) mm (vs. 20-35 mm), corollas lanate glandular (vs. hairy glandular) and lobes of lower lip of corolla acute (vs. rounded).

O. tunetana has been found as parasite, only, on wild specimens like *Plantago albicans* but, regarding its affinity with *O. aegyptiaca*, it may a future parasitic weed.

1.3. Other species like *O. purpurea* Jacq. a very rare taxon, growing on *Achillea odorata (Compositae)*, could also be a future parasite on ornamental plants like *Achillea* spp.

2.1. In the Section *Orobanche*, several are the broomrapes important as agricultural parasites. *O. cumana* Wallr. [=*O. cernua* subsp. cumana (Wallr.) Soó; *O. cernua* var. cumana (Wallr.) Beck] a very harmful species, growing exclusively on *Helianthus annuus* (sunflower), can be considered an allocthonous species from East

Europe and Central Asia and introduced in Spain with the culture of the sunflower. By the other hand, *O. cernua* Loefl. is a close relative of the former. Some botanists (Bonnier, 1911-1935; Chater & Weeb, 1972; Pignatti, 1982) have even considered both taxa like synonymous (*O. cernua=O. cumana*). *O. cernua* is a plant of the Mediterranean area, sensu lato, and native of the Iberian Peninsula, growing on several *Compositae: Artemisia barrelieri, A. campestris subsp. glutinosa, A. gallica, A. herba-alba* and *Launea lanifera.*

The differences between *O. cernua* and *O. cumana* are shown in Table 1 (cf. Pujadas and Thalouarn, 1998)

2.2. *O. crenata* Forskal, (=*O. speciosa* DC.) is another very important host of agricultural plants, mainly *Leguminosae*. Distributed in the Mediterranean area: South Europe, North Africa, South West Asia and Canary Island. Parasite on traditional crops as *Vicia faba, V. sativa, Lens culinaris, Pisum sativum, Cicer arietinum, Daucus carota* subsp. sativa or *Carthamus tinctorius*. It also grows on wild plants that can be considered as secondary hosts and as hosts reserve of *O. crenata*. Some of these wild plants are: *Anagyris foetida, Coronilla scorpioides, Lathyrus clymenun, L. latifolius, L. tingitanus, Lupinus albus, Medicago ciliaris, M. orbicularis, M. sativa, Psoralea bituminosa, Trifolium campestre, T. tomentosum, T. repens, T. alexandrinum, Vicia lutea, V. sativa (Leguminosae), Conopodium capillifolium, Daucus carota subsp. maximus, Eryngium campestre, Foeniculum vulgare subsp. piperitum, Tordylium maximum (Umbelliferae), Calendula arvensis, Chrysanthemum coronarium, Coleostephus myconis, Lactuca serriola, Pallenis spinosa, Picris echioides, Sonchus oleraceus (Compositae).*

It is characterised by its showy spikes, flowers with calyx 13-18 mm, with segments free bidentate; corolla 18-28 mm glandular pubescent, white, the lips often with lilac veins, lips divergent, large, not ciliate; filaments inserted 2-3(4) mm above base of corolla, hairy at base with glandular hair at apex; anthers brown, glabrous or subglabrous.

2.3. *O. foetida* Poiret is an important agricultural plant as parasite, in Tunisia, growing on *Vicia faba* and *Cicer arietinum* (Kharrat et al. 1992). Distributed in South West of Europe (Portugal, Spain) and North West Africa (Morocco, Algeria, Tunisia, Libya). In the South of Spain grows on *Leguminosae* wild species, mainly on *Astragalus lusitanicus, Scorpiurus muricatus, Trifolium angustifolium, Lotus* sp. and *Melilotus* sp.

It is a dark reddish plant; flowers with calyx segments very long acuminate; corolla 12-23 mm dark purplish-red, inside and outside, narrow and +/- straight, lower lip not ciliate; filaments inserted (1) 3-7 mm above base of corolla, stigma yellow.

It can be considered as a potential dangerous parasite plant of the *Leguminosae* crops in South of Spain.

O. foetida subsp. broteri Guimaraes (= *O. foetida* var. lusitanica Cout.) has a shorter corolla of (11) 13-15 mm and filaments inserted 1-2 mm above base of corolla.

It can also be considered as a potential parasite of pastures, because it has been found on Trifolium repens and *T. pratense* in the wild.

2.4. *O. minor* Sm, largely distributed in West, South & Middle Europe, South West Asia, North Africa & West Africa.

Found on the cultivated garden plant *Gazania rigens* (*Compositae*) and on many pasture wild plants as *Trifolium tomentosum, T. repens, T. angustifolium, T. stellatum, T. hirtum, T. arvense, T. subterraneum,* and *Vicia lutea, (Leguminosae).*

Characterised by calyx 7-12 mm, segments equally or unequally bidentate or entire; corolla 10-18 mm, generally glandular-pubescent, white usually tinged with dull violet distally or yellow; filaments inserted 2-3 mm above base corolla, poorly hairy at the base or subglabrous; anthers purple; stigma purple.

O. hederae Duby, a very close plant to *O. minor,* also belonging to the Section Minores. From West, South and Middle Europe, and North West Africa (Morocco, Algeria). It can be considered as an important parasite of ornamental plants, growing on *Hedera helix* cultivated in gardens or in the wild.

It is a yellowish to reddish brown plant, with calyx 10-15 mm with segments free, entire rarely unequally bifid; corolla 10-22 mm, glabrous to subglabrous, yellow to dull cream tinged distally with reddish purple, corolla tube somewhat inflated below, gradually narrowed to the mouth, nearly straight; lower lip not ciliate; filaments more less glabrous rarely somewhat hairy below, inserted 3-4 mm above base of corolla; stigma yellow.

2.5. *O. latisquama* (F.W. Schultz) Batt., distributed in the West Mediterranean Region (Portugal, Spain, Morocco, Algeria).

It has been found exclusively on wild plants of Rosmarinus (Rosmarinus officinalis, R. eriocalyx, R. tomentosus), but could also be a possible parasite on R. officinalis cultivated in gardens.

It is characterised by its calyx 15-20 mm, segments connate in proximal quarter, entire (rarely bifid); corolla 25-30 mm, galeate, subglabrous with subsessile glandular hairs, pale yellowish tinged with purple towards the lips, upper lip subentire, lower lip equally three-lobed, not ciliate; filaments hairy inserted 8-12 mm above base of corolla; anthers villous purple; stigma white.

Other *Orobanchaceae* species.

The genus *Cistanche* Hoffmanns & Link differs from the genus *Orobanche* L. in its campanulate calyx with five equal obtuse lobes; corolla scarcely bilipped and nearly regular with five subequal patent lobes.

Cistanche phelypaea (L.) Continho is a plant distributed in South Portugal, South and South East Spain, Crete, Canary Islands and North Africa to Middle East.

It grows on woody *Chenopodiaceae*, generally wild plants: *Atriplex halimus, Hammada articulata, Salsola genistoides, S. oppositifolia* and *Sarcocornia fruticosa.* Important as parasite on *Atriplex halimus* cultivated as a fodder plant in the South East of Spain (Murcia province).

It is a stout plant, glabrous; flowers with calyx 13-18 mm, very big corollas 30-40 (60) mm bright yellow; anthers and filaments hairy.

Conclusion.

Several species are very well known as important parasites of agricultural plants: *Orobanche ramosa, O. cumana, O. crenata,* whilst *O. foetida* can be considered as a neophyte parasite of important crop in Tunisia.

Some species are parasites of ornamental cultivated plants, at present, as *O. nana, O. minor, O. hederae*, and others can be considered as potential host of ornamental cultivated plants as *O. purpurea* and *O. latisquama.*

O. minor is also a frequent parasite of pasture plants, *Cistanche phelypaea* is a neophyte on fodder plants and *O. foetida* and *O. foetida* subsp. broteri can potentially be parasites of pasture plants.

Less important, but potentially aggressive can be considered *O. mutelii, O. tunetana* and *O. cernua.*

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TABLE 1: Morphological difference between *Orobanche cernua* Loefl. and *O. cumana* Wallr.

	O. cernua	*O. cumana*
Stem (cm)	15-32	(35) 40-65
Inflorescence (cm)	6-17 (23)	(17) 22-30 (38)
Inflorescence type	Dense, rarely lax at the base	Lax, sometimes dense at the apex
Calyx (mm)	6-10	(5) 7-9
Calyx segments	Bidentate to unequally shortly bifid, sometimes entire	Entire, sometimes the basal flowers unequally deeply bifid
Corolla (mm)	15-18	(17) 19-22
Corolla position	Erect to erect-patent	Erect-patent to patent
Corolla dorsal line	Arched forward geniculated	Curved forward –downward inflected
Corolla, limb colour	Dark blue to violet	Pale blue
Anthers	Glabrous or sparsely pubescent at the base of the suture line	Hairy at the base of the suture line

EATING BROOMRAPE?

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Broomrape (*Orobanche crenata*) is a major constraint for legume cultivation in large areas of the Mediterranean and East Asia. Several control strategies have been employed, such us delayed sowings, long rotations, trap and catch crops, hand weeding, solarization, herbicides, biological control and genetic resistance, but all without unequivocal success. The methods used are either not feasible, uneconomic, hard to achieve or result in incomplete protection. Thus, integration of several control measures is more desirable. Even when the technology for the control of broomrape is available, its implementation is impeded because of the low input crops that are infected in countries where advanced cropping systems may not always be in place.

Another potential strategy would be to change the end uses, so that broomrape would not be just a constraint for legume production, but a desirable product in its own right. Dioscorides reported that the stalks of broomrape can be eaten as a potherb similar to asparagus. In the Puglia region of Italy, broomrape is considered a tasty vegetable and has been eaten since Roman times. The young broomrape shoots are picked from the faba bean orchards and can be bought in local greengrocers (Bianco, 1993; Ditonno and Lamusta, 1997). Broomrape is also eaten in areas of Morocco. There are many recipes for cooking broomrape, and as can be seen below, they are likely to satisfy a variety of different tastes.

Hand weeding is not economical. Further, if the pulled shoots are keep in the field they are able to produce seeds, so the weed problem persists. If the practise of harvesting young shoots for human consumption were to spread, this would not only help to reduce the damage to the host plant and to reduce the brommrape seed-bank in the soil, but also provide an additional source of incomes to small holders. This approach would not only provide a sustainable means of control but also contribute towards economic sustainability.

Recipes for cooking broomrape (after Ditonno and Lamusta, 1997, N. Greco, pers. comm.): collect young tender shoots before flowering. Cut at soil level and wash in water. Cook in any of the following ways:

1.- Raw broomrape: 200 g broomrape, olive oil, wine vinegar, salt.
Cut very tender shoots in thin slices and add oil, salt and vinegar. They are typically served as a garnish with faba bean purée.

2.- Broomrape salad: 500 g broomrape, mint leaves, garlic, wine vinegar, olive oil, salt.
Boil and drain the broomrapes. Add the mint, vinegar, salt and oil. Mix and serve warm or cold.

3.- Broomrape with faba beans: 500 g broomrape, 500 g young faba bean seeds, olive oil, salt.
Boil till tender the broomrape and the faba bean seeds. Drain and add the oil and salt.

4.- Fried broomrape: 500 g broomrape, 200 g wheat flour, 2 eggs, oil, salt.
Blanch the broomrapes and then leave them in fresh cold water for at least 24 hours, changing the water from time to time. Make a soft dough with the wheat flour, eggs, water and a little salt. Coat the broomrapes with the dough and fry in hot oil until golden-brown. They can be served warm with slices of lemon.

5.- Broomrape "alla Parmigiana": 500 g brommrape, 200 g wheat flour, 4 eggs, olive oil, cream cheese, 0.5 l tomato juice, 120 g grated cheese.
Fry the broomrapes as described above. Arrange them on an oven tray in layers alternating with grated cheese, the boiled eggs chopped in slices, cream cheese and tomato juice. Cook in the oven at 180°C for 40 minutes or until golden brown.

6.- Oven cooked broomrape: 500 g broomrape, 2 eggs, salt, olive oil, garlic, grated cheese.
Blanch the broomrapes and allow them to stand in fresh water at least 24 hours, changing the water from time to time. Put the broomrapes on an oven tray. Add salt, oil, garlic and grated cheese and mix thoroughly. Then, add some more cheese, the whipped egg, oil and a few spoons full of water. Cook in the oven at 180°C for 40 minutes to golden brown.

6.- Broomrape stored in vinegar:
Blanch the broomrapes. Drain and wait until cold. Put them in a bottle and fill with vinegar. Close the bottle hermetically.

7.- Broomrape stored in oil:
Blanch the broomrapes Drain and wait until cold. Place them in a bottle along with salt, mint leaves and garlic, and fill the bottle with diluted vinegar. Allow to stand

for one day. Remove the vinegar and fill the bottle with oil. Close the bottle hermetically. The broomrapes can be eaten after a few days or stored for several months.

8.- Broomrape omelette: 200 g broomrape, 2 eggs, salt, olive oil

Blanch the broomrapes and allow them to stand in fresh water at least 24 hours, changing the water from time to time. Fry them lightly. Beat the eggs, add the broomrapes and mix thoroughly. Pour in a frying pan with a bit of oil and cook one side, turn it over and cook the other till golden brown.

In addition, many other recipes can be produced introducing the broomrape as a new ingredient in other typical recipes from different countries. It is perhaps ironic that for some of the recipes both the host plant and the parasite are major ingredients. This not only reflects the evolution of the host-parasite association, but also demonstrates how the farmers adapted their diet to include both plants. It is known that broomrape reduces the faba bean yield, but the final dry matter of the faba bean plus the broomrape is commonly maintained, so for a farmer eating both the broad beans and the broomrapes the infection would not represent a loss of food. Those growing field beans for animal feeding would have a reduction in seed yield, that would be only somewhat reduced by removing the broomrape shoot, but the main gain would be the reduction of the seed bank in the soil. Broomrape can be eaten by the cattle, but the practise is not recommended as the seeds remain viable, thus providing an additional way of spreading the plant.

Alternative uses of broomrape could include the pharmaceutical and cosmetic industries. A few species in *Orobanchaceae* have been used in medicine. Their medicinal properties have been known since the 17th century. Great broomrape (*O. major synonym O. rapum-genistae*) was prescribed as a medicinal herb through most of Europe "as a remover of stone in the bladder and kidneys and a provoker of lustry urine". It was usually administered decocted in wine. Applied externally the juice was used to treat wounds and ulcers. The decocted flower spikes were used as a wash for "cleansing the skin" and "for fleckles, black or blue spots or pushes thereof". *O. virginiana* was used to control diarrhoea, ulcers and gangrene (Mitich, 1993).

We can, therefore, envisage broomrape as a desirable by-product of the legume crops, used for industrial and for culinary purposes. But being even more imaginative, depending on the acceptance of these products and the prices they could get, they could exceed the importance of the host crops themselves, with the legumes acting as a substrate needed for broomrape production. Such production could take different forms. One could be the intensive production of young shoots that could be sold for fresh consumption or processed and canned like asparagus, for instance.

Another could be the production of dry shoots to be used by the pharmaceutical industry. Commercial use of parasitic plants is not novel, given the importance of sandalwood (*Santalum album*) and the research that has been undertaken to find suitable hosts for the trees and the best ways of encouraging growth. Other example is the root hemiparasitic tree quandong (*Santalum acuminatum*), whose fruits are used in jam making in Australia. The fruits of Exocarpus cupressiformis and *Acanthosyris falcata* are edible. In some parts of Africa the roots of the parasitic *Cynomorium coccineum* are used as seasoning. The *Balanoforaceae* have been used in Java to extract wax for candles. The rhizomes of *Ammobroma* (fam. *Lenoaceae*) were an important source of food for the American natives (Heywood, 1985).

If *Orobanche* species were to have commercial uses, then legume hosts would need to be selected for susceptibility, allowing a maximum supply of resources to the parasite to ensure maximum broomrape production. Broomrape breeding would also be required to meet the particular requirements of the end user. There is big genetic variation within broomrape populations, so just a simple selection could yield interesting results in terms of increasing dry matter production, content of desirable substances for medicinal or cosmetic uses, or enhanced taste, flavour or palatability for culinary purposes.

The success of such an approach would depend on the acceptance of these products by the markets. To meet that demand we need to show and spread the benefits of using broomrape and to develop the technology for broomrape production, collection and distribution at a reasonable price. Many requirements to meet, but the prospect is certainly food for thought.

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