Parasitic flowering plants – from botanical curiosity to antibiosis

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The term parasites frequently refers to microorganisms, fungi, worms or insects. However, parasites have also developed among higher plants, with various degrees of dependency on the sap of their host plants. Currently, more than 3000 species of flowering plants are known being parasitic; these live from the assimilates, nutrients and water of their hosts. Continued adaptation of host and parasite takes place during the course of common evolution and ensures the development of both partners in the natural habitat. However, the frequent occurrence of host plants in agricultural production systems results in improved reproduction conditions for the parasite and in a high infestation probability for the crop, depressing yields of pulses and pasture legumes, oil crops and vegetables.

Although the holoparasitic plant Orobanche is distributed world-wide, only the losses in South and East Europe, in North Africa and West Asia are significant. Some particularly damaging Orobanche species occur in the Mediterranean region and in Western Asia and parasitize important cash and food crops. O. aegyptiaca and O. ramosa attack mainly legumes and Solanaceae, such as eggplant, potato, tobacco, tomato. O. crenata and O. minor cause most damage in the cool-season food and feed legumes, while O. cumana causes great damage to sunflower. In the Mediterranean basin and W-Asia around 16 million ha are endangered by Orobanche - about 1.2% of the world arable land. Other regions with similar climatic conditions (Australia, South America (Chile), USA) have also been invaded posing an actual or potential risk to crop production.

The main regions of distribution of the hemiparasite Striga are located in the arid and semiarid tropics. These regions have a predominant vegetation of natural savanna, semi-evergreen, and deciduous rain forest. Economic loss to agricultural production is caused by three species: S. asiatica and S. hermonthica parasitize exclusively members of the Poaceae family corn, sorghum, millet, and rice in low-potential areas. S. gesnerioides is specialized on dicotyledons, such as cowpea and bambara groundnut. In Africa and S-Asia grain production is endangered on millions of hectare of land affecting the livelihoods of millions of people. Although the level of Striga infestation and damage is increasing, farmers rarely adopt Striga control methods. This is because of lack of awareness and knowledge about Striga biology among farmers, national extention services and local government authorities. Hence, little support and activities are provided by the organizations. So far, parasitic weeds have not benefitted from the enormous advances in the control of pests, weeds and diseases. This is largely due to the fact that both host and parasite are flowering plants tightly affiliated, which require approaches that make distinction between both partners.
Parasitism in members of the genus *Orobanche* has led to a simplification in their morphology and therefore to a reduction in features used to distinguish species. The intrinsic taxonomic difficulties in *Orobanche* are further compounded by the fact that important differential characters can be observed only with difficulty, or not at all, in dried specimens, and the features used to distinguish species are poorly defined. Polyploidy, interspecific crosses, hybridization among different ploidy levels, parthenogenesis, chaotic meiosis and mitotic abnormalities also contribute to the taxonomic difficulties in the group.

In order to clarify genetic relationships and to obtain a better taxonomic classification we have sequenced and analysed chloroplast *trnD - trnT* region of a total of 25 *Orobanche* taxa, 17 taxa belonging to the section *Orobanche* (*O. alba, O. almeriensis, O. amethystea, O. ballota, O. cariofilacea, O. cernua, O. clausonis, O. crenata, O. cumana, O. gracilis, O. gracilis ssp. sprunerii, O. gracilis ssp. austrohispanica, O. haensleri, O. heredae, O. icterica, O. laserpiti-sileri, O. teuvrii*), and 8 belonging to the section *Trionychon* (*O. lavandulacea, O. mutelii, O. nana, O. olbensis, O. rosmarinus, O. schutzii, O. tunetana*).

Phylogenetic analyses were implemented with PAUP* 4.0b10 using maximum-parsimony (MP), neighbor-joining (NJ), and maximum-likelihood (ML) methods. The pattern of interspecific variation revealed is in agreement with previous taxonomical studies of *Orobanche*. The differences between sections *Trionychon* and *Orobanche* observed in this study, seem to corroborate the taxonomic classification established on the basis of morphological traits as well as RAPD markers.
Experimental data strongly suggest the existence of several pathovars in *Orobanche ramosa L*.

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Genetic variations among 11 *O. ramosa* samples collected in different regions of France were studied using intersimple sequence repeat (ISSR). ISSR patterns showed specific markers indicating the existence of two pathovars: \( pvT \) (for tobacco) and \( pvC \) (for canola). Broomrape plants collected on tobacco, tomato, hemp and *Polygonum fagopyrum* belong to the \( pvT \). Broomrapes collected on Canola (winter rape) and *Buddleja* belong to the \( pvC \).

Cross infestations were performed in pot experiments in the greenhouse using four varieties of Canola, four varieties of hemp and *O. ramosa* seeds of the two pathovars (\( pvC \) and \( pvT \)). The seeds of *O. ramosa* (\( pvC \)) led to high levels of infestation in Canola and only a very low level of infestation in hemp. Inversely, seeds of *O. ramosa* \( pvT \) provoked a strong infestation in hemp while canola was almost unattacked.

Moreover ISSR patterns are being performed using *O. ramosa* samples collected in several other European countries. The pathovar \( T \) was found in *O. ramosa* parasitising tomato (in Romania) and tobacco (in Spain). *O. ramosa* samples collected on tobacco or tomato in Italy showed ISSR patterns different from those observed in \( pvC \) or \( pvT \).

So, the genetic variability in *O. ramosa* may be large and this species could contain several pathovars showing different levels of virulence.
Assessing genetic variability in *Striga hermonthica* and *S. aspera* by RAPD and SCAR analysis

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In Africa, *Striga* is the greatest biological constraint on food production. *Striga hermonthica* (Del.) Benth. and *Striga aspera* (Willd.) Benth. are recognized as separate species, but due to their close morphological similarity it is difficult to distinguish them, especially in East Africa. Both species can be crossed artificially and hybrids seem to be more virulent than their parents. Although it is unknown whether hybridisation occurs naturally in the fields, it is worthwhile to investigate this possibility in view of the potential high virulence.

In this study *Striga* from several geographic regions of Africa and a few F1 and F2 hybrids were tested. A genetic analysis was performed by means of DNA profiles derived from genetic polymorphism RAPD-PCR markers using AMOVA (Analysis of Molecular Variance). Parental and hybrid populations were separated with the help of AMOVA and the Arlequin software package.

The amount of variation due to within population polymorphism was 67.09% (*S. hermonthica*), 50.13% (*S. aspera*) and 58.22% (hybrids). The amount of variation due to among population polymorphism was: 32.91% (*S. hermonthica*), 49.87% (*S. aspera*) and 41.78% (hybrids).

After comparing the RAPD data of the populations a phylogenetic tree was constructed using UPGMA. Four major clusters of *Striga* populations were found: *S. hermonthica* from W. Africa, *S. aspera* and two *S. hermonthica* groups from East Africa. The hybrids appeared to cluster to their maternal parent..

Two SCAR markers were developed and in combination it was possible to distinguish the two species *S. aspera* and *S. hermonthica*; furthermore *S. hermonthica* populations from West Africa could be distinguished from those from East Africa, at least the populations we had analysed. With the help of these markers it was also possible to distinguish the hybrids from each other.
Defense gene expression in host roots infected by Orobanche species

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The infection of Arabidopsis thaliana roots with the holoparasite Orobanche ramosa represents a useful model for a molecular study of the host plant response to a parasitic plant attack. Thus, we developed an in vitro co-culture system, allowing us to investigate the expression pattern of some host plant genes selected among genes known to be involved in metabolic pathways and resistance mechanisms activated during several plant-pathogen interactions: ethylene, isoprenoid, phenylpropanoid, and jasmonate pathways, oxidative stress responses and PR proteins. A non-targeted study based on a suppression subtractive hybridization strategy was also used to identify genes that were induced two hours after placing O. ramosa seeds near A. thaliana roots. Infestation will not start before the seventh day. The kinetic gene expression was assayed from 1h to 7 days after O. ramosa germinations were placed. Proteins encoded by these genes are also involved in A. thaliana defense pathways: signal transduction, pectin methylesterase inhibition, detoxification of reactive oxygen species, jasmonate-dependent pathway and cell wall reinforcement. From these studies, no salicylic acid dependent defense has been detected whereas jasmonate- and ethylene-dependent pathways were induced. This work also revealed that signals emitted by O. ramosa-germinated seeds trigger early defense molecular reactions in host roots, even before parasite attachment. In order to assay the specificity of the response, we carried out crossed experiments using both O. ramosa and O. cumana on Arabidopsis and sunflower (susceptible and resistant genotypes) roots. Preliminary results indicated that the molecular response of the host depends on the parasite virulence as well as on its degree of resistance.
Biology and control of parasitic weeds: *Striga* and *Orobanche*

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One aspect of recent research has been the identification of novel sources of resistance to *Striga hermonthica* in a wild relatives of maize. The susceptibility of one such wild relative, *Tripsacum dactyloides*, and a *Zea mays*-T. *dactyloides* hybrid to *S. hermonthica* infection has been determined. *S. hermonthica* development is arrested after attachment to *T. dactyloides*. Vascular continuity is established between parasite and host but there is poor primary haustorial tissue differentiation on *T. dactyloides* compared with *Z. mays*. Partial resistance is inherited in the hybrid. *S. hermonthica* attached to *Z. mays* was manipulated such that different secondary haustoria could attach to different hosts. Secondary haustoria formation was inhibited on *T. dactyloides*, moreover, subsequent haustoria formation on *Z. mays* was also impaired. Results suggest that *T. dactyloides* produced a signal that inhibits haustorial development: this signal may be mobile within the parasite haustorial root system.

A second project has taken a different approach to identifying resistance to *Striga* in maize. Together with colleagues in CIMMYT, a large population of maize which contained mutator type transposable elements was developed. Mutator elements preferentially insert into coding regions of the genome, knocking out gene function, these are therefore optimal for transposon-tagging. Eight thousand F2 families from the transposon-tagged maize population were screened in the field in western Kenya during 1998 and 1999. Interesting families were identified as those which had segregating *Striga*-free plants (no emergence) within the family. Twenty three families have been identified which have no/low emergence of *Striga*. All these families displayed 1:3 segregation for the *Striga* free trait (25% *Striga* free, 75% *Striga* emergence), suggesting that that a single recessive mutation is responsible for the observed phenotype. Laboratory observations on one of these lines demonstrated that the growth of *Striga* was severely impaired resulting in a low incidence of emergence. A field screen of F3 families derived from the 23 interesting F2 lines confirmed the interesting phenotype in a number of entries.

A third project has addressed the identification of tolerance in maize cultivars to *Striga asiatica*. The screening of a large number of maize cultivars from Africa revealed that one cultivar, CML395, demonstrated tolerance to *S. asiatica* infection under both laboratory and field conditions (in Zimbabwe) at a low nitrogen supply. This cultivar produced high grain yields, despite being infected by *S. asiatica*. Typical yields in the field were in the range of 2162 to 3645 kg/ha. Nitrogen forms (inorganic and organic) did not differ in terms of their impact on maize and *S. asiatica*. Timing of nitrogen was not critical for *S. asiatica* tolerance in cultivars CML395 and R201. Of interest was that cultivar CML395 produced high grain yields on-farm in the absence of added nitrogen (966 to 1837 kg/ha). Current research also focuses on using molecular genetic approaches to understand the basis of resistance and susceptibility to *Striga* and *Orobanche* using model systems, including *Arabidopsis thaliana* as well as cereal models.
Germination of broomrape seeds

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The genus \textit{Orobanche} contains about 100 species of obligate root parasites of which some are important agricultural pests because they parasitise legumes, crucifers, tomato, sunflower, hemp and tobacco particularly in Southern and Eastern Europe, North America, East Africa, The Middle East and the Indian subcontinent (Joel, 2000). The first critical step in their life cycle - germination of the seeds - is tightly regulated by specific chemical signals exuded from the roots of the host plants, known as germination stimulants (GS). The germination stimulants are active in extremely low concentrations from $10^{-7}$ to $10^{-15}$ M (Joel, 2000). Our group is interested in the biosynthesis of these germination stimulants in host roots (Bouwmeester et al., 2003). Therefore, to be able to sensitively detect these compounds, we have studied the effects of the preconditioning temperature and preconditioning period on the sensitivity of parasitic weed seeds to the synthetic germination stimulant GR24. Results will be discussed.

In addition, nothing is known about the regulation of the biosynthesis of these compounds in the roots of the host species, except that all the compounds so far identified have been hypothesised to belong to the sesquiterpenes lactones (Bouwmeester et al., 2003).

We focus on the biosynthesis of the germination stimulants for \textit{O. ramosa} in the roots of arabidopsis, for \textit{Striga hermonthica} in the roots of maize and sorghum and will start work on \textit{O. crenata} germination stimulant formation in legume roots. In our attempt to pick-up GS-biosynthesis related genes we are screening mutant collections such as an arabidopsis activation tagging transposon mutant collection (generated at PRI) for phenotypes with altered germination stimulant production.

\textit{In vitro} grown plants and hairy root cultures are used to study the effect of inhibitors and putative intermediates and substrates of pathways. Parallel to these biochemical approaches to elucidate the biosynthesis, we also follow a molecular approach by testing existing mutants in a number of biosynthetic pathways for altered induction of germination.


Recent experience in *Orobanche* Control by Suicide Germination

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Stimulation of suicide germination by synthetic strigol-analogues appears as a promising specific and environment-compatible method for *Orobanche* control.

Field trials in former years have shown differing but promising results in the control of *Orobanche ramosa*. During the season 2003 field trials were carried out in South Baden, Germany, on an experimental tobacco field, which is heavily infested with *Orobanche ramosa*. Treatment with a Nijmegen-preparation was carried out four weeks before transplanting tobacco. In the control plots 49 days after transplanting (dat) all tobacco plants were parasitized by *Orobanche*, while on the treated plots only few *Orobanche* spikes had developed. 63 dat (14.07.2003) all tobacco plants on the control plots were surrounded by bushels of *Orobanche*, while on treated plots 20% of the tobacco plants were infected with one *Orobanche* each. Four weeks later, when the tobacco was almost harvested, the treated plots also showed increasing number of *Orobanche*. However, the tobacco on treated plots appeared strong and healthy with a plant height of 100-120 cm, while on control plots the plants were stunted with small leaves and a height of less than 60 cm. The results will be discussed in more detail.

Trials with glyphosate, even applied in moderate concentration, again showed high phytotoxicity and partly total damage of the tobacco plants.
Changes in the sensitivity of parasitic weed seeds to germination stimulants

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The first critical step in the life cycle of parasitic weeds – germination of their seeds, is regulated by specific chemical signals exuded by the roots of host plants. The germination stimulants are active in extremely low concentrations from $10^{-7}$ to $10^{-15}$ M (Joel 2000). Our group is interested in the biosynthesis of these germination stimulants in host roots (Bouwmeester et al., 2003). Therefore, to be able to sensitively detect these compounds, we have studied the effects of the preconditioning temperature and preconditioning period on the sensitivity of parasitic weed seeds to the synthetic germination stimulant GR24. Preconditioning at an optimal temperature (21 °C for Orobanche cumana and 30 °C for Striga hermonthica) rapidly released dormancy and increased the sensitivity with several orders of magnitude. After reaching maximum sensitivity, prolonged preconditioning rapidly induced secondary dormancy, i.e. decreased sensitivity of O. cumana and S. hermonthica to GR24. The rapid change in sensitivity of preconditioned seeds to germination stimulants during prolonged preconditioning was particularly clear at low concentrations of GR24. GR24 in higher concentrations (0.1 and 1 mg.l⁻¹) usually induced high germination of O. cumana and S. hermonthica regardless of the preconditioning period. This rapid change in the sensitivity of Orobanche and Striga seeds to germination stimulants during prolonged preconditioning was not reported before. This is without doubt due to the high concentrations of germination stimulants that are often used in laboratory experiments, and which do not allow us to precisely estimate the actual dormancy status of preconditioned seeds. Our results show that parasitic weed seeds are highly sensitive to the germination stimulant for a short period of time only and then relatively quickly enter into secondary dormancy. The similar germination pattern of S. hermonthica seeds preconditioned for prolonged periods of time under laboratory and field conditions suggests that the mechanism observed is of ecological significance.


Sanitation and quarantine policies need to be adopted in Europe

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The effect of broomrapes on agricultural production may increase when the agricultural systems become more vulnerable to the parasite due to climate changes. This may be true not only in Mediterranean and East-European countries but also in areas that are presently free of the parasite. Nevertheless no serious measures are in effect in Europe to prevent the entry of broomrape seeds and to stop their spread from infected areas.

Sanitation measures need to be constructed and enforced, and quarantine policies should be discussed and imposed in order to prevent future damage.

The following are proposed key activities that need to be adopted:

1. Map the infected areas;
2. Develop diagnostic tools;
3. Operate monitoring services/authorities;
4. Develop and enforce sanitation and quarantine policies;
5. Decide on broomrape management policies;
6. Prepare educational programs and literature;
7. Nominate officers to be in charge of the broomrape management program;
8. Coordinate policies internationally.

COST action 849 seems to be the best forum for the preparation of comprehensive broomrape management scheme and quarantine policies for Europe and the Mediterranean.
Evaluating integrated management strategies for *Orobanche* and *Striga*

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Methods of evaluating and comparing integrated control strategies for the parasitic weeds (*Striga* and *Orobanche*) will be considered. Since some interventions only offer benefits to succeeding crops, it is suggested that a good starting point is that of the effects of interventions on depletion of the natural soil seed bank in the absence of seed shedding. Differences also need to be considered from the perspective of the farmers’ criteria of reduced infestations in susceptible crops and ultimately in terms of economics. In one example, effects of rotations and fertility improvements on *Striga hermonthica*, will be shown. Germination depends on the distance of the parasite’s seeds from the roots of the plant producing germination-stimulating exudates. Interestingly, therefore, depletion is greater for seeds located within than between crop rows. Over two seasons seedbank depletion exceeded 90% in the most effective treatments, which included a trap crop in the first season. By contrast, depletion was only 46% with continuous cropping with a susceptible crop and combining this with the common practice of allowing seed shedding, increased the seedbank by 270%. Seed losses were however very much slower than the rapid short-term seed losses reported in some other studies and precautions are needed if naturally occurring seed banks are not to be used. Similar analyses may be suggested for *Orobanche* based on life cycle models.
Chlorsulfuron resistant transgenic tobacco as a tool for broomrape control

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Broomrape (Orobanche ramosa L.) is the most important parasitic plant that infests tobacco (Nicotiana tabacum L.). Chemical treatment of the soil is not effective and crop rotation is not acceptable to solve this problem because of the long viability period of Orobanche seeds in the soil. Application of systemic herbicides in the field with herbicide resistant tobacco could be a successful tool for broomrape control.

Several tobacco cultivars were transformed with a mutant ahas3R gene for resistance to the herbicide chlorsulfuron (Glean®, DuPont). Transformed plants were selfed and the segregation of resistance was followed in the next generation. The efficiency of the herbicide was demonstrated in greenhouse and field trials. An Orobanche/tobacco growth system was used in order to prove the lethal effect of the herbicide to the attached broomrape plants.
Orobanche ramosa control in tomato.

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In Greece some 30% of tomato area, or 15,000 ha are thought to be infected by Orobanche ramosa to a varying and increasing degree with losses averaging 25%. Control if any (on 60% of farms) is by hand-pulling only. In island Crete, however, where the problem is localized within 200 ha, control is achieved by solarization (80%) or methyl bromide (20%).

In order to find a solution to this problem we used several herbicides. Chlorsulfuron, glyphosate and imazaquin were the most effective of them. All three herbicides reduced the dry weight of Orobanche shoots and underground attachments more than the number of these organs. However, in pot experiments chlorsulfuron applied at 5.0 g a.i./ha gave 100% control of the number and dry weight of emerged broomrape shoots and underground attachments. This herbicide was the most effective against broomrape as well as the least toxic to the crop. Under field conditions chlorsulfuron applied at 10 g a.i./ha controlled broomrape emergence by 88%, while when it was applied, twice (5+10 g a.i./ha) it gave complete control of broomrape but delayed crop maturity. Imazaquin applied at 9.25 g a.i./ha did not effectively control broomrape in pot experiments. Higher rates of this herbicide increased its effectiveness but reduced crop yield. In field experiments, the same rates and even higher were less effective against broomrape and less toxic to the crop. Glyphosate applied at 45 g a.i./ha did not control broomrape. Its effectiveness was increased at the rates of 90 and 180 g a.i./ha, but not consistently. This herbicide at all rates was not toxic to the crop grown either in pots or in field conditions. The results of this study indicated that chlorsulfuron could effectively control broomrape in tomato, while imazaquin and glyphosate may have some potential uses for this purpose. However, more research is needed to determine the timing of the herbicide application when the parasite is in the most sensitive and the crop in the most tolerant stage.

Since last years, research has been directed towards the use of transgenic crops against parasites, we decided to investigate whether Orobanche could be controlled efficiently in transgenic glyphosate-resistant tomatoes. Greenhouse experiments were conducted using the line 1232 of processing tomatoes, engineered with the plasmid pMON894, which contained a gene from Escherichia coli, coding for an altered glyphosate-resistant enzyme: 5-enolpyruvyl-shikimate 3-phosphate synthase. Non-transgenic tomato plants (UC82B) were also used. Monsanto kindly provided seeds of both tomato lines. Glyphosate was applied at 180, 270 and 360 g a.i./ha, in three periods (20, 30 and 20+50 days after tomato transplanting). The results showed that the two higher rates of glyphosate were the most effective against Orobanche in all periods of application. None of the glyphosate rates reduced shoot dry weight in transgenic tomatoes whereas the higher rates were toxic to the non-transgenic tomatoes. These results clearly indicated that the use of glyphosate-resistant transgenic plants could be a solution to the Orobanche problem because it allows the use of higher rates of this non-selective herbicide.
Control of *Orobanche ramosa* by glyphosate in tomato

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A trial to control *Orobanche ramosa* L. was carried out in a tomato field naturally infested by the parasitic species, near Altamura (Southern Italy). Glyphosate was applied at 4 doses (9 - 18 - 36 and 54 g ha$^{-1}$) after tomato transplanting, up to 4 applications at weekly intervals. The preliminary results showed that: a) in every treated plot a reduction of parasitized tomato plants was observed, compared to the control; b) the herbicide appeared particularly effective when applied four times at the lowest dose; c) glyphosate caused a slight phytotoxicity when distributed four times at the highest rate; c) the few broomrape spikes emerged had a low percentage of viable seeds.
Impact of *Orobanche ramosa* to the yield of tomato fruits in the southwest of Slovakia

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In the southwest of Slovakia, tomato is grown as a field plant for the production of juice and paste. *Orobanche ramosa* infection was found to influence the tomato plants. Together 100 tomato plants infected by *O. ramosa* and 100 non-infected plants were compared one month before harvest of fruits. Average number of *O. ramosa* sprouts per one infested tomato plant was 9.54. Average number of fruits per non-infected tomato plant was 41.78, and average number of fruits per infested plant was 16.85. During evaluation in August, average non-infested plant yielded 15.86 fruits with the diameter of 0.1–2.0 cm, 15.84 fruits with the diameter of 2.1-4.0 cm and 10.08 fruits with the diameter more than 4 cm. Average number of fruits on infested plant was 4.57 (0.1-2.0 cm), 7.02 (2.1-4.0 cm) and 5.25 (more than 4 cm), respectively. Mathematic calculation showed, that there was an equivalent of 37.93 fruits with the diameter of 3 cm per one non-infested plant and 17.31 fruits per one infested plants, respectively.
Control of *Orobanche* sp. in melon and watermelon crops in Cyprus

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In Cyprus, broomrape (*Orobanche ramosa* L. and/or *O. aegyptiaca* Pers.) parasitizes on several crop plants, including cabbage (*Brassica oleracea* L. var. *capitata* L.f.), celery (*Apium graveolens* L.), tomato (*Lycopersicon esculentum* Mill.), eggplant (*Solanum melongena* L.), melon (*Cucumis melo* L.), and watermelon (*Citrullus lanatus* Thunb.), causing severe crop damage and yield reduction. Work at the Agricultural Research Institute has led to the formulation of strategies that successfully control the parasite in broad beans, cabbage, celery, tomatoes and eggplants, while trials for its control in melons and watermelons are under way. For the control of broomrape in melon and watermelon, different amounts of olive pumice (byproduct of olive oil processing), and soil mulching with black polyethylene sheet are tested in a heavily infested field. Watermelon seedlings used in this experiment are grafted on a cucurbit rootstock which is resistant to soil-borne diseases. Pumice was incorporated into the field soil at 60, 30, 7.5, and 2.5 l per running meter of a 30 cm furrow prior to planting melon and watermelon seedlings grown in modules. Also, black polyethylene sheet was applied just prior to planting melon and watermelon seedlings to plots where 7.5 and 2.5 l pumice was added. Covering the soil with black polyethylene gave in all cases the best results in reducing orobanche infestation and increasing yield. The 30 and 60 l amounts of pumice caused severe toxicity to both melon and watermelon. Nevertheless, the reduction of orobanche was similar to that observed in treatments covering the soil with black plastic. Trials will be repeated in 2004 and a further treatment concerning the use of grape pumice will be included in the experiment. Should this method prove successful it may be applied in extensive crops such as potatoes, peas etc.
Control of broomrape (*Orobanche crenata* Forsk.) in narbon bean (*Vicia narbonensis* L) by glyphosate.

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The efficacy of glyphosate on broomrape control on narbon bean (*V. narbonensis*) was studied the season 2003-2003 under field conditions at “Alameda del Obispo” experimental farm in Córdoba, southern Spain, using the susceptible line IFVN 586 Sel 2393 from ICARDA. The experimental design was a fully randomised block with three replicates. Each treatment consisted in a four-rows plots (4 x 1.5 m\(^2\)) treated with glyphosate at 0 (control, only water), 35, 67 and 201 g. a. i./ ha (0, 0.5, 1 and 3 times the recommended rates on faba bean (Mesa-García *et al.*, 1984; Mesa-García and García Torres, 1985)), in a double treatment, the first application at early flowering of the crop, when first tubercles were observed. The application was repeated ten days later. Number of emerged broomrpes per host plant was counted at crop maturity in 15 central plants, and grain yield determined.

Glyphosate reduced broomrape emergence at all concentrations studied, with increasing levels of control with the increase in the glyphosate rate. Treatments at rates 2 (67 g.a.i./ha) and 3 (201 g.a.i./ha) caused evident chlorosis and rolling of apical leaves affecting yield. In contrast, no macroscopically visible signs of phytotoxicity were observed when glyphosate was applied at rate 1 (30 g.a.i./ha), resulting in a significant increased of yield. Therefore, this herbicide appear to have some potential for broomrape control in narbon bean.

**Acknowledgements**

This study was supported by PIA-03-052 from Dirección General de Investigación y Formación Agraria y Pesquera de la Junta de Andalucía.


Broomrape control in Romania

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Sunflower broomrape (Orobanche cumana Wallr.) causes severe yield and quality losses in Romania, each year. Infested fields are being abundant and for the farmers it is difficult growing sunflower in these fields.

The development of sunflower hybrids resistant to the new races of this parasite is therefore essential, as is the incorporation of resistance genes into standard lines that have good production properties and high GCA (general combining ability). We detected sources of resistance in accessions of Helianthus annuus, H. divaricatus, H. nuttallii, H. grosseserratus, H. tuberosus (Marinescu A. and Maria Păcureanu Joia, 1998) and a crossing programme has been started to exploit that resistance in sunflower breeding (Păcureanu et al., 2002).

Resistance ought to be integrated with other control methods to ensure an adequate protection and its durability. Herbicides have proven effective in controlling Orobanche sp. in sunflower (Garcia – Torres et al., 1992). Resistance to herbicides exists in sunflower germplasm, this being exploited in breeding (Miller, 1999). Sunflower inbred lines resistant to herbicides are developing at ARDI Fundulea Romania, in order to obtain resistant hybrids. So, the herbicides, such as glyphosate, imazapic, benuronmetil and other ones can be applied for broomrape control.

Prevention of attack is an important way to control broomrape in Romania. Soil management, control of used seed lots, sowing date or simply destroying broomrape in infested fields may to ensure a protection against this parasite.

Integrated control of crenate broomrape in pea

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O. crenata is potentially the major constraint for pea cultivation in the Mediterranean area and Middle East. The actual low levels of broomrape resistance would suffice to retard broomrape attachment and/or development in normal years, but would not be enough in conducive years and in heavily infested fields. We have detected sources of resistance in accessions of pea as in wild Pisum (Rubiales \textit{et al.}, 2003) and a crossing programme has been started to exploit that resistance in pea breeding. However, available sources resistance are only incomplete and of complex nature, what makes resistance breeding a difficult task. Pea crop should, therefore, be protected with other control measures. Good broomrape control can be achieved in faba bean by glyphosate at low rates. This is however not possible in pea as it is far more sensitive to the herbicide even at lower rates than recommended on faba bean. Pea tolerates well pre- and post-emergence treatment of other herbicides suitable for broomrape control such as imazethapyr. However, imazethapyr treatment was less effective in the earlier sowing dates. Even when a double treatment might by effective, farmers are reluctant to adopt the technology, as field pea in the area is a low input crop of low yield, with little margin for treatments. Because of the extreme difficulty of controlling broomrape, prevention is of great importance.

Alternative control measures have been approached. A delayed sowing till middle January might account for a significant reduction in broomrape infection with acceptable yield. This might be however, highly dependant on weather conditions. Foliar applications of BTH (activator of systemic acquired resistance) provided a significant reduction of broomrape infection under controlled conditions. However the technology needs to be improved before it can be recommended to farmers. Perspectives from experiences with biocontrol with Phytomyza and search for tolerance are discussed.


Toxins from pathogens of parasitic plants

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Most of the pathogens isolated by parasitic plants and proposed as possible biological control agents belong to the genus *Fusarium*, a genus well known to produce a wide array of toxins, with different biological activities, chemical structures, mechanisms of action, specificity with respect to plants, environmental impact, and stability. Since those fungal metabolites have been intensively studied mainly due to the risks posed to human and animal health when these toxins accumulate in agricultural commodities and are absorbed through nourishment, usually they are thought to be a risk. Often very promising mycoherbicides have been discarded by final evaluation processes just because in vitro they produce powerful and dangerous toxins. The evaluation of the “real” risk should be ascertained by considering the global environmental impact, i.e., determining the exact production of those metabolites when fungi are formulated, or when they are applied against, and grown on, target weeds; the toxicity to non-target organisms; the stability of toxins in planta or the absorption by soil particles; and the risk of water drift. On the other hand, toxins could be used to directly or indirectly enhance the efficacy of weed biocontrol agents, depending on their biological and chemical characteristics, through: 1) the selection of organisms overproducing toxins; 2) their synergistic use with biocontrol agents; 3) their use as biomarkers; 4) their use as sources of natural herbicides.
So what if transgenic hypervirulence changes host range of a biocontrol agent? We need not to jump to conclusions.

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We must face the truth that inundative biocontrol has been an abject failure in controlling weeds in row crop agriculture – and this includes the biocontrol of Orobanche. There is only one inundative biocontrol agent marketed for row crops – for the control of a weed in rice paddies, where the humidity is not lacking. Classical biocontrol for Orobanche in most of the COST area is by definition impossible, as Orobanche is indigenous, so inundative biocontrol is imperative, so should we give up? The main reason for the lack of success is the evolutionary balance between host and indigenous candidate agent chosen for biocontrol – if the biocontrol agent was sufficiently virulent to give the level of weed control demanded by the farmer – both the weed and the biocontrol agent would have become extinct. Virulence can be enhanced transgenically and the biocontrol agent cultivated industrially – to prevent extinction. A forma speciales of Colletotrichum coccodes that Alan Watson and his group showed was highly specific to Abutilon theophrasti, not attacking any of 200 other crop and weed species. The organism was not commercially acceptable as it infected only cotyledons, needed as large inoculum and a long dew period. We have demonstrated that when it was transformed with the nep1 gene, encoding a protein toxin, the organism killed through the three leaf stage, with less inoculum and a shorter dew period, but alas it also infected tomato and tobacco when their foliage was sprayed in the same manner as the weed (Nature Biotechnology 20:1035-1039, 2002). Does this mean that the transformed organisms and the whole are of research must be abandoned because of a change in host specificity? Farmers are used to using selective herbicides, which are analogous to selective biocontrol agents, and the above agent did not attack cotton, the major crop where the weed is a problem. The herbicides used in cotton also kill other crops, and farmers still use them, usually successfully, precluding spray to prevent lawsuits from neighbors. Indeed, we have designed mechanisms to lower drift of such agents such as using asporogenic mutants that cannot drift via spores, and engineering genes that would render the organism less able to attack other crops (Trends in Biotechnology 19:149-154, 2001). It is also unwise to assume that the same gene will render another organism pathogenic to tomatoes and tobacco. Indeed, we transformed the nep1 gene into a Fusarium arthrosporioides that attacks some Orobanche species. The transformed organism produces the toxin in liquid culture, yet grew on the rhizoplane of tomato roots, without affecting the crop. It was hypervirulent to Orobanche trying to grow on the tomato, killing it far quicker, and leaving fewer unkillled individuals than the wild type.

Thus, it seems that we can conclude that a transgene encoding a hypervirulence factor that changes the host range of one species of biocontrol agent, will not necessarily change the host range of other species; and even if it does change host range, it can be contained.

“The conclusion you jump to may be your own” (James Thurber).
The efficacy of a mixture of fungi to control Egyptian and sunflower broomrape

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Broomrapes (Orobanche spp.) are the most troublesome weeds in Israel causing severe yield and quality losses. The most important species are Egyptian (O. aegyptiaca) and sunflower (O. cumana) broomrape. Recently Fusarium solani (Fs) was isolated in Israel from Egyptian broomrape diseased inflorescences. Isolates of F. solani (ET4) and F. oxysporum (FT2) were isolated in Italy from diseased inflorescences of O. ramosa and were found to be pathogenic to O. ramosa in pot experiments. The fungus F. oxysporum f.sp. orthoceras (Foo) was isolated from O. cumana diseased inflorescences and caused disease symptoms only on O. cumana. In the present project the efficacy of each of the isolates and their mixtures was tested on sunflower broomrape on sunflower and Egyptian broomrape on tomato.

Sunflower – Soil inoculation with ET4 and Foo caused severe disease symptoms and mortality of sunflower broomrape, reaching mortality level of 89 and 83%, respectively. Fs and FT2 were less effective and reduced the number of inflorescences emerging above soil level by 60 and 43%, respectively. Soil inoculation with a mixture of Fs with either ET4 or FT2 had not effect on sunflower broomrape mortality level. However, inoculation with a mixture of Fs and Foo reduced by 83% the sunflower broomrape dry weight attached to the roots of the sunflower plant at the end of the experiment as compared with 42% reduction when the soil was inoculated only with Foo. A mixture containing all four isolates was less effective then Fs + Foo but more effective then Foo alone.

Tomato – ET4 and Fs demonstrated moderate activity toward Egyptian broomrape parasitizing tomato roots. However, their mixture was highly pathogenic causing high disease index and mortality, reduced considerably broomrape dry weight and the number of inflorescences above the soil. Foo and FT2 did not cause any disease symptoms on Egyptian broomrape plants. Addition of each of these isolates to Fs antagonized its pathogenic activity. Inoculating the soil with a mixture of all four fungi was less effective then the mixture of ET4 and Fs.
Natural antagonists of *Orobanche* spp. in Tunisia with potential as biocontrol agents

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A survey for natural antagonists (insects, fungi and rhizobacteria) of parasitic weeds of the genus *Orobanche* was carried out during the growing season of 2000 and 2001 in the major legume cropping areas in Tunisia. *Phytomyza orobanchia* (Diptera: Agromyzidae) and *Smicronyx cyaneus* (Coleoptera: Curculionidae) were found to attack *O. crenata* Forsk. and *O. foetida* Poiret in all surveyed areas. Damage of both insects under natural conditions has been evaluated. One hundred and five fungal isolates were obtained from infected *Orobanche* underground stages with *Fusarium* spp. being the most prevalent. *Fusarium oxysporum* and *F. culmorum* showed promising control of *Orobanche* in screening experiments. Bacteria were isolated from the rhizosphere of faba bean as well as from diseased *Orobanche* underground stages or suppressive soil samples. Of 351 bacterial isolates obtained, 337 were screened for antagonistic activity against both *Orobanche* species. Both, *Orobanche* seed germination stimulating bacteria and *Orobanche* growth inhibiting bacteria have been selected which showed a significant antagonistic activity against the parasitic weed. Most promising isolates were *Pseudomonas fluorescens*, *Ps. marginalis* and *Ralstonia pickettii*. 
To determine the effect of insect species feeding on dodders (*Cuscuta* spp.) under field conditions in Slovakia, 36 locations infested with dodders were irregularly observed during 2002. Weevils from the genus *Smicronyx* (Coleoptera: Curculionidae) were found to be the principal natural enemies of dodders in Slovakia. Together 756 specimens were collected during the study. *Smicronyx jungermanniae* (Reich) was the most abundant accounting for 93.4% of the total weevils reared. *S. coecus* (Reich) and *S. smreczynskii* Solari were rare and accounted only for 5.6 and 1.0%. Larvae of *S. jungermanniae* and *S. smreczynskii* caused stem galls on *C. europaea* and *C. campestris* as well as seed destruction on *C. epithymum* and *C. europaea*. On the other hand, larvae of *S. coecus* were found in flowers and seeds of *C. epithymum* and *C. europaea* only. Infestation of seed capsules, caused by *Smicronyx* spp., ranged between 0 and 100%. The species prevents flowering and fruiting on *Cuscuta* spp. and could be significant biological control agents of these parasitic weeds.