Proceedings of the meeting “Integrated control of bromrape”

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1. Progress in biological control of *Orobanche* in Italy

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A national project funded by the Italian Ministry of Scientific Research entitled “Biological control of *Orobanche* spp. using phytopathogenic fungi and their toxic metabolites” started 2 year ago. Field surveys led to isolate from *Orobanche ramosa* seeds and plants more than 50 fungal strains, belonging to 16 different species, 12 out of them to the genus *Fusarium*, with 17 *F. oxysporum* strains and 18 *F. solani*. All the selected strains were tested for pathogenicity and virulence using an adapted plastic bag system. Fungi were applied to small tubercles and symptom appearance was observed daily up to 2 weeks. The strains were ranked into four groups, depending on their effects on tubercles, ranging from ineffective to highly virulent. 9 isolates resulted to be very interesting, being able to cause quickly necrosis of tubercles, with subsequent decay. They were further assayed in pots, applying the spore suspensions to the soil surface. Few of those strains confirmed their interesting activity, strongly prevent *Orobanche* shoot emergence. They allowed to obtain health and better growing tomato plants, used as broomrape host, sensibly reducing the number of the subterranean tubercles and without producing any disease symptom on tomato roots. Assessment of fungal specificity both with regard to other *Orobanche* species and to crop plants is now in progress. For the production of toxic metabolites, the selected strains were grown using both liquid and solid media; the cultures were then extracted with organic solvents, analysed by chromatography and biologically assayed on broomrape seeds, with the aim to find new bioactive metabolites inhibiting seed germination. The purification and biological characterization of at least two metabolites both from liquid and solid cultures are in progress. The content of fusaric and dehydrofusaric acids in liquid cultures of *Fusarium* strains was also ascertained and quantified by HPLC, with the aim to use those metabolites as biomarkers to select the most active strains.
2. Fungal pathogens from naturally infected *Orobanche* found in Greece

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The occurrence of fungal pathogens of broomrape (*Orobanche* spp.) in Greece has never been reported. Therefore, a survey has recently been initiated in various parts of the country to locate naturally infected *Orobanche* plants. Inflorescences and stems of broomrape collected from heavily infested crops were examined for symptoms indicative of fungal infections. Although, broomrape inflorescences have not yet been found to suffer from fungal pathogens, symptoms associated with primary fungal infections have been observed on stems. A fungal strain isolated from infected stem tissue of a species of *Orobanche* parasitizing broad bean (*Vicia faba*) plants, seemed to be associated with deterioration observed at the base of the stem of the parasitic plant, causing no damage to the host. The isolate belongs to the genus of *Fusarium* and grows readily on liquid or solid substrates. Pathogenicity tests and phytotoxicity bioassays are in progress in order to determine the host-specificity of the isolate and to evaluate the phytotoxic potential of extracts obtained from the cultures of the fungus.
New approaches are necessary to control parasitic weeds of the genus Orobanche. The fly Phytomyza orobanchia (Diptera, Agromyzidae) is particularly suitable for biological control since it feeds oligophagous only on Orobanche species. In total, of the 140 Orobanche spp. described, the occurrence of P. orobanchia is reported from 21 species.

The use of P. orobanchia in biocontrol of Orobanche is based on inundative releases at the period of Orobanche emergence. The larvae of P. orobanchia mine in Orobanche shoots and capsules and intervene at the sensitive reproduction stage of Orobanche. Hence, the reduction of Orobanche seed production prevents supplementary infestation and dissemination. The advantage of this control approach is its compatibility to all crop/Orobanche associations and that it can easily be combined with other control methods.

In northern Morocco, the application of P. orobanchia in biocontrol of Orobanche spp. has been tested from 1995 until 1999. Under natural conditions, 48.9% of Orobanche seed capsules are infested by P. orobanchia. P. orobanchia is parasitized by 9 hymenopterous species, but the total parasitization rate does not exceed 8.9% on an average. For field releases of P. orobanchia adults, a formula for the calculation of the fly number per hectare based on the Orobanche infestation level has been developed. Inundative releases of P. orobanchia in field cages have shown that the natural efficiency of P. orobanchia can be increased considerably. Only 5.3% of viable seeds have been produced in comparison to 62.0% without inundative releases. Seeds are directly destroyed by the mining activity of P. orobanchia larvae as well as indirectly by the feeding damage of shoot tissues causing a degeneration of seed capsules. In highly infested fields (> 200 Orobanche shoots per m²), an increase of the Orobanche seed bank in the soil could be still observed after inundative releases. In low to medium infested fields, releases of P. orobanchia alone are sufficient to reduce the Orobanche seed population to an acceptable level. An integrated control with tolerant and/or resistant cultivars, the combination with mycoherbicides or other control methods is proposed.
4. Phytomyza orobanchia Kalt. on different species of Orobanche in Slovakia

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A survey was carried out in 2001 to determine the occurrence and distribution of the agromyzid Phytomyza orobanchia Kalt. (Diptera: Agromyzidae) on broomrapes (Orobanche spp.) in southwestern and northwestern Slovakia. Two major Orobanche species are common in these areas of Slovakia: O. ramosa L. parasitizing mainly tobacco (Nicotiana tabacum L.) and tomato (Lycopersicon esculentum Mill.) in southwestern Slovakia and O. flava F. W. Schultz attacking butterbur (Petasites sp.) and rarely coltsfoot (Tussilago farfara L.) in northwestern Slovakia. Although, there is no clear overlapping of the both areas (O. ramosa in southwestern and O. flava in northwestern), the percentage of natural infested plants by P. orobanchia was similar. Shoots of the broomrapes were examined at 5 localities (tobacco fields) in southwestern Slovakia and 5 localities (with natural occurrence of butterbur) in northwestern Slovakia. The agromyzid was present at 100% of the localities sampled. Of 300 O. ramosa plants examined, 79% were infested, similarly as 85% of 300 O. flava plants. Climatic conditions did not influence significantly the abundance of P. orobanchia. O. ramosa grows in lowland at an altitude of 100-300 m a. s. l. and O. flava grows in highlands (altitude 600-950 m a. s. l.).

The results validate that P. orobanchia feeds on hosts of the genus Orobanche and its distribution is related to the natural occurrence of plants of Orobanche spp.

Our findings are the first confirmed records of P. orobanchia on O. flava.
5. *Fusarium solani* as a possible agent for broomrape control

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In our previous experiments we have found that *F. solani,* isolated from diseased broomrape plants, was destructive to all *Orobanche aegyptiaca* developmental stages and prevented the damage caused to tomato plants by the parasite.

In the present study we tested the host range of the fungus by inoculating soil with a suspension containing hyphae and spores of *F. solani.* No reduction in seed germination or foliage development of bean, pea, vetch, cotton, cucumber, tomato, melon and sunflower could be observed. The infection with *F. solani* increased seed germination and the total biomass of bean, pea, sunflower and cotton.

Crude extract of the fungus growth media demonstrated toxic activity, inhibiting Egyptian and sunflower broomrape seed germination and causing necrosis on inflorescences. The same bioassay didn’t produce any visible symptoms on the leaves of the above-mentioned crops.

Application of *F. solani* together with *F. oxysporum* f. sp. *orthoceras* to soil containing sunflower broomrape seeds provided full protection to the sunflower plants growing in these pots. The combined effect of the mixture of the two fungi was much more pronounced than the inoculation with each fungus alone. The total number of broomrape plants in the pots treated with *F. oxysporum* f. sp. *orthoceras* was less than in the pots treated with *F. solani.* However, *F. solani* completely prevented the emergence of broomrape inflorescences above soil level, while a few inflorescences emerged in the pots inoculated with *F. oxysporum* f. sp. *orthoceras.* The number of the parasites per plant in the pots inoculated with mixture of the two fungi was lower and all of them died at earlier developmental stages, than in the pots inoculated with each fungus alone. All fungal applications caused no damage to the crop plant and inoculation with *F. solani* induced an increased growth response.
6. The Phytomyza status in Israel

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A crucial problem with Orobanche is the ability of a single plant, which escapes control, to develop Orobanche seed bank in soil. Rapid proliferation of seed populations leads to high rates of infestation shortly after first invasion of single broomrape seeds into uninfested fields. Therefore, any attempt to control this parasitic weed should also tackle seed production. Phytomyza orobanchia has the advantage of being specific to broomrape. Its larvae feed on Orobanche ovules and young seeds, and also on stem tissues. The fly has advantages as a biocontrol agent against Orobanche also because it is self mobile and therefore provides flexibility in application with regards to the exact location of the parasitic weed. P. Orobanchia was reported for Israel, but has so far not been used in this region as a biocontrol agent. Nevertheless in a survey of broomrape infected agricultural fields in various parts of Israel we found that the fly is highly effective in reducing seed production of various broomrape species in spite of the fact that it has not been introduced by man, and in spite of the presence of Phytomyza-specific parasitic wasps.
Organisms proposed for biocontrol of major weeds in arable row-crop agriculture have not met expectations due to an evolutionary balance between microorganism and weed, even when the mycoherbicide is used at “inundative” levels. If the virulence of a mycoherbicide matched that of chemical herbicides, the weed and the microorganism would have become extinct. Soil active biocontrol agents for *Orobanche* must contend with competing soil microorganisms, as well as the weed, while not affecting the crop host. Greater virulence can be achieved by transferring factors to the microorganism, tipping the evolutionary balance in favor of the microorganism, knowing that if it is truly successful, there will be a requirement for seasonal applications, as with a chemical herbicide. There are advantages to hypervirulent host-specific organisms that disappear after killing their host; they do not leave a residue in the soil, decreasing the likelihood of evolution of host change. *Fusarium oxysporum* Schlecht (FOXY) and *F. arthrosporioides* (Fide) Wollenw. (FARTH) pathogenic on *Orobanche aegyptiaca* were transformed with two genes of the indole-3-acetamide (IAM) pathway leading to indole-3-acetic acid (IAA) to attempt to enhance virulence. Transgenic FOXY lines containing both the tryptophan-2-monoxygenase (*iaaM*) and indole-3-acetamide hydrolase (*iaaH*) genes produced significantly more in IAA than the wild type. Indole-3-acetamide accumulated in culture extracts of FOXY containing *iaaM* alone. FARTH containing only *iaaM* accumulated IAM and an unidentified indole. Some transformants of FOXY expressing only the *iaaM* gene also produced more IAA than the wild type. Sub-threshold levels of transgenic FOXY expressing both genes and FARTH expressing *iaaM* were more effective in suppressing the number and size of *Orobanche* shoots than the wild type on tomato plants grown in soil mixed with *Orobanche* seed. The same genes enhanced the activity of a *Colletotrichum coccodes* on *Abutilon theophrasti*, but only when the organisms were applied with the tryptophan substrate needed for the activity of the transgenic enzymes. Adding tryptophan can be a failsafe mechanism for a foliage applied mycoherbicide – it is hypervirulent only in the presence of tryptophan, and is a wimp without it. The utility of using methyltryptophan and fluorotryptophan to select for tryptophan-overproducing *Fusarium* mutants of the transformants is being tested to preclude the need for added tryptophan. Stimulating an auxin imbalance enhanced pathogen virulence. Because of the fear of spread of transgenic organisms, and the additional fear that genes will introgress from an *Orobanche*-specific *Fusarium* to a crop pathogen, we plant to institute the two-fold level of fail safe mechanisms recently proposed: a. to generate asporogenic mutants that can be applied only as formulated mycelia; and b. to flank the hypervirulence transgene with transgenes that would be neutral vis a vis *Orobanche*, but would be deleterious to crop pathogens (anti-melanin genes, anti-appressoria genes, etc.).

Parasitic plants of the genus *Orobanche* cause serious damage to several crops in the Mediterranean Basin, Central Asia, Arabia and some African Countries. The biology of the weed and the close host-parasite interaction, are the main obstacles for the achievement of a control using chemical and cultural strategies. For this reason, biological control is considered at the moment one of the most promising systems to control the weed. Among the entomofauna associated with *Orobanche* spp., two seed/stem feeders, the fly *Phytomyza orobanchia* and weevil *Smicronyx cyaneus*, are the most promising candidates.

The great potential of these insects to control the weed, due to the impact on the seed bank, can be amplified when used in combination with pathogens. In fact, insects with endophytic behavior show a capability to carry secondary damage in the target weed. The possibility to be vectors of pathogenic transmission can be enhanced by using bait traps associated with spores of the selected fungi.
9. Integration of fungal toxins with pathogens

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Many plant pathogens are able to produce phytotoxins, bioactive metabolites having different chemical structure, mechanism of action, specificity respect to the plants, biological activity, environmental impact, stability. Most of the known and studied phytotoxins are produced by plant pathogens due to the interest to understand diseases caused by agriculturally important toxigenic fungi. In the last decades, thank to the increased interest in weed biocontrol using pathogens, many weed pathogenic fungi have been discovered and studied. Many of them belong to important toxigenic genera, such us \textit{Fusarium}, that are known to produce a range of chemically different phytotoxic compounds, i.e. fusaric acid, fumonisins, beauvericin, enniatin, moniliformin and trichothecenes. These compounds possess a variety of biological activities and the effects they are able to produce include necrosis, chlorosis, growth inhibition, wilting, inhibition of seed germination and effects on calli. Depending on their biological and chemical characteristics, these bioactive metabolites could be of great help in integrated management of parasitic plants, and different approaches could be used:

1) study of role of toxins in plant-pathogen relationship: the knowledge of meaning of phytotoxins in plant disease, mechanism of action and biosynthetic pathway could be of great help in drawing weed bio- and integrated control strategies;

2) direct application with weed pathogens: phytotoxins have different spectra of action with respect to the producer pathogens; their use with pathogens could increase herbicidal efficacy of the fungus, in terms of pathogenicity, virulence, speed of action or selectivity;

3) discovery of new classes of natural herbicides: many phytotoxins have toxicological properties that render them good frames to create new classes of natural and safe herbicides, or that could be synthesized, or modified in their functional groups changing their biological activity; with regard to parasitic plants, considering that stimulation of seed germination by host root exudates is one of the key phase of their cycle, the use of inhibitors of germination could be an interesting approach. At this regard, some \textit{Fusarium} toxins proved to be highly active preventing inhibition of seed germination. For example fusaric acid, one of the commonest toxin can completely suppress germination of \textit{Striga hermonthica} seeds when used up to $10^{-6}$ M.

4) use as biomarkers: correlating toxin production with other physiological or biotechnological pathogen characters could permit an easier \textit{in vitro} selection of the most useful strains. Preliminary observations on several strains of \textit{F. oxysporum} isolated from \textit{Orobanche ramosa} seem to demonstrate a positive correlation between \textit{in vitro} fusaric acid production and virulence of strains.
10. *Nep1* literally transforms sleeping mycoherbicides into Rambos

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The needed hypervirulence of mycoherbicides can be achieved by transferring factors encoding toxins to the microorganism, tipping the evolutionary balance. To use low molecular weight toxins would entail engineering multi-gene pathways into the mycoherbicide. We therefore chose the *nep1* gene encoding a 24 kD “necrosis eliciting protein” isolated by Bryan Bailey at the USDA. Virulence was increased 9 fold, and the requirement for a long dewpoint decreased by introducing *nep1* to an *Abutilon theophrasti*–specific, weakly mycoherbicidal strain of *Colletotrichum coccodes*. The parent strain was at best infective on juvenile cotyledons of this intransigent weed. The transgenic strain was lethal through the three-leaf stage, a sufficient time window to control this asynchronously germinating weed.

The *nep1* gene was originally isolated from a *forma speciales* of *Fusarium oxysporum* and engineering the gene into the *Fusarium oxysporum forma speciales* specific to *Orobanche* spp\(^1\) was without greater effect than the wild type. PCR analysis showed that the *nep1* gene was present in the wild-type strain, perhaps silenced in such a way that the transgene is not expressed. Conversely, the gene is not present in the *Orobanche*-specific *F. arthrosporioides*, and the *nep1* transgene greatly improved the virulence of this strain, with little or no damage apparent from the phytotoxic product to the crop host. This suggests that the toxin may be produced locally on contact with *Orobanche*, and not when the fungus is growing in the rhizosphere of the crop. The results strongly indicate that this line of research be continued to greenhouse testing, and with proper fail safe mechanisms and regulatory approvals, to the field.

11. Formulation and application of a potential mycoherbicide against *Orobanche cumana*

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*Fusarium oxysporum* Schlecht. f. sp. *orthoceras* (Appel & Wollenw.) Bilai was isolated in Bulgaria from diseased shoots of *Orobanche cumana* Wallr. The fungus was found to attack all developmental stages of the parasitic weed including the seeds and it also proved its bioherbicidal efficacy under field conditions when propagated on organic substrates and applied to the soil at rates between 800 and 1200 kg/ha. To reduce the amount of inoculum needed for satisfactory *Orobanche* control, microconidia or chlamydospore-rich biomass of the fungus were matrix-incorporated in granules made from wheat-flour and kaolin (‘Pesta’). The formulated material efficiently controlled the parasitic weed in greenhouse trials, irrespective of the incorporated inoculum type. Pre-planting soil incorporation of 1 g formulated material per pot (13x13x13 cm, 2 kg soil) led to a reduction of *Orobanche* emergence of up to 80 % and a high disease incidence on the remaining shoots, especially after a split-application. Comparing wheat-kaolin granules with different inocula regarding their storability at room temperature, granules with chlamydospore-rich biomass had a better shelf-life than the microconidial preparations. In granules containing air-dried chlamydospore-rich biomass, about 80 % of the initial Colony Forming Units (cfu) were recovered after 8 months of storage.

In a first field trial carried out in Israel, the influence on *Orobanche* emergence and the level of disease was lower compared to the pot experiments and a slightly better performance of granules containing chlamydospore-rich biomass became obvious. The soil population of the fungus in the plots treated with the higher dosage of granules was about 1 to 2 x 10^5 Colony Forming Units (CFU) per g soil in 5 to 10 cm depth one week after application but decreased to less than 10 % of the initial numbers within two months. In 10 to 20 cm depth, the initial population was lower but the decrease over time was less pronounced. In further investigations, the main factors responsible for the poor survival of the pathogen and the low level of *Orobanche* control in the field have to be clarified. First results indicate that high temperatures lower the efficacy of the biocontrol agent. Biotic and abiotic soil factors may also play an important role regarding the performance of the biocontrol agent, indicated by an enhanced reduction of *Orobanche* number and dry matter as well as higher fungal population counts in a soil high in organic matter compared to a loamy sand; an effect that could be reduced by sterilization of the latter.
Rhizobacteria were isolated in Tunisia from the rhizosphere of faba bean as well as from diseased Orobanche underground stages or soil samples. Three hundred and thirty-seven isolates were screened for antagonistic activity against *Orobanche crenata* Forsk. and *O. foetida* Poiret.

The isolates were screened first on *Lactuca sativa* seedlings. The *Lactuca sativa* bioassay yielded 37 isolates (11\%) with a growth inhibition activity and 18 isolates (5\%) with a stimulatory effect. The remaining isolates (84\%) were growth-neutral to lettuce seedlings. Both growth-inhibiting or growth-promoting isolates were further evaluated for their antagonistic activity against *Orobanche*. Some selected rhizobacteria characterized as DRB (deleterious rhizobacteria) greatly reduced the infestation with *Orobanche* when screened in root chambers or pot trials and have no deleterious effect on the host plant. Those bacteria with a stimulatory effect were tested on the *Orobanche* seed germination. In the preliminary tests some isolates significantly increased the germination of *O. crenata* and *O. foetida* seeds. Some isolates showed also a pronounced plant growth promoting activity when tested on lentil.
13. Impact of seasonal effects on parasitic weed model prediction

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Seasonal variation includes abiotic factors such as temperature (mean, minimum and maximum), soil water content and potential, soil nutrient status and soil atmosphere among other variables.

It is useful to clarify first what are the objectives of the modelling. For example:
♦ Academic (various sub-objectives may be included here);
♦ Practical short-term simulations and predictions
♦ Practical long-term simulations and predictions
♦ Aids to decision support.

Other questions on seasonal factors may include:
♦ How significant are abiotic factors in modifying different stages of the life cycle?
♦ To what extent in modelling can we ignore these factors (it is often done!)
♦ To what extent can we use long-term weather data as inputs to our models and to what extent do we need accurate forecasts?
♦ To what extent must we consider interactions with microorganisms and other biotic factors? (E.g toxins and germination stimulants are clearly important).
♦ Perhaps most importantly, how do responses vary within and between populations?
Examples will be shown illustrating that what is usually being modelled is the seed-to-seed variation in response.

Some examples of seasonal variation in depletion of soil seed banks are also mentioned using both predicted and observed data. For example, predictions of loss of viability of seeds of Orobanche in Table 1 are based on temperature and humidity.

Table 1 Prediction of loss of viability of O. aegyptiaca under farm conditions in a tomato growing place in Eritrea in a full year (from Kebreab & Murdoch (1999). Grain Legumes (23): 8-9)

<table>
<thead>
<tr>
<th>Season</th>
<th>Mean Temperature (°C)</th>
<th>Relative Humidity (%)</th>
<th>Period (months)</th>
<th>Predicted loss of viability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early dry season</td>
<td>20</td>
<td>53</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Late dry season</td>
<td>26</td>
<td>40</td>
<td>5</td>
<td>2.5</td>
</tr>
<tr>
<td>Rainy season</td>
<td>25</td>
<td>Imbibed</td>
<td>2</td>
<td>33</td>
</tr>
<tr>
<td>Cumulative total loss</td>
<td>12</td>
<td></td>
<td></td>
<td>38.5</td>
</tr>
</tbody>
</table>
14. Implications of nitrogen relations for parasite growth models

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The impacts of parasitic weeds on their hosts are mediated by a number of biotic and abiotic variables, and among these soil fertility is a major factor, in particular nitrogen availability. The ways in which N impacts on parasitic weeds and their hosts will be discussed and key points that can be manipulated, as part of an integrated management programme, will be identified. The ways in which this information can be used in a modelling context will be considered, particularly in the light of existing models examine nitrogen (and other nutrients) in crop-weed systems.
15. Orobanche-weeds relationships: an important aspect of broomrape control.

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For most part of the countries where Orobanche ramosa L. is spreading, weed flora is very important and diversified (1,2,3,4). For example, it includes 838 taxa in Morocco (1) and 1200 in France (2); which represents for each country 20% of the national flora of vascular plants. For the preoccupant weed flora (about 15% of the specific effective), the major botanical families are: Asteraceae, Poaceae, Fabaceae, Brassicaceae, Caryophyllaceae, Apiaceae, Polygonaceae and Papaveraceae. Like the different species of Orobanche and other phytoparasites, these weeds are parts of the agronomic landscape and cannot be forgotten in broomrape control.

The interaction between O. ramosa and plant crops have been well studied (5), but only few data are available concerning the weed host range of this parasite. Such informations could be useful in an integrated program. Weeds may serve as “trap” plants depleting orobanche seeds from soil or “tank” plants increasing broomrape seed-bank in soil.

In this study, we have tested the response of 36 weed species (mainly annuals) belonging to 20 botanical families (nearly all of them are above-mentioned). Most of them have a large distribution in Europe (France, Germany, Spain,..) and/or countries where O. ramosa sets an agronomic problem (Morocco for example).

Our study evidenced a wide range of weeds responses to O. ramosa. Three main groups have been distinguished:

i/ susceptible species:
the parasite succeeded in full development. We observed emergence and seed production. The most susceptible ones were: Sonchus oleraceus L., Senecio vulgaris L., Galium aparine L. ssp. aparine, Capsella bursa-pastoris (L.) Medic. ssp. bursa-pastoris, Coronopus didymus (L.) Smith, Sonchus asper (L.) Hill and Raphanus raphanistrum L.. These weeds could contribute to increase O. ramosa seed-bank and thereby make up a secondary infestation centre. It is noticeable that five of them belong to botanical families including O. ramosa- susceptible crops as oilseed-rape in Brassicaceae and sunflower in Asteraceae.

ii/ resistant species:
two sub-groups can be distinguished:
* in the first one, weed species supported viable attachments but no production of any emergence. A slow growth or a late fixation of the parasite could explain this incomplete development. Most of them presented few fixations, whereas Lapsana communis L. had a great number of attachments with an important necrosis at stage 1 or 2 and in afew cases, a little apical bud was observed on stage 2.
* for the second, weed species supported more or less broomrape fixations but in all cases necroses appeared at stages 1 and 2. Different resistant levels were observed by histological studies: endodermic barrier (Avena sativa L. ssp. sterilis (L.) De Wet), encapsulation layer (Ammi majus L.), thickening of host vessels (all the species), vessels occlusion (Ammi majus, Solanum nigrum L ssp. nigrum), cell wall polyphenolic impregnation (Anagallis arvensis L. ssp. arvensis, Solanum nigrum ssp. nigrum). These processes lead to parasite weakness or death. These weeds could be considered as “trap” plants. It is interesting to note that most of them belong to botanical families of agronomic importance (Solaceae, Asteraceae, Apiaceae and Poaceae). Some of these weed species could be good models for resistance mechanism study.
immune species: these weeds did not allow broomrape attachment. Lack of germination stimulants in roots exudates could be the main cause of this immunity. A very early resistance mechanism cannot be excluded.

This survey of the O. ramosa weeds host range contributes to a better understanding of weed impact on O. ramosa distribution. These informations could be useful in an integrated program. In addition, some resistant weeds like Solanum nigrum ssp. nigrum, Lapsana communis, Datura stramonium L., Cichorium endivia L. ssp. pumilum (Jacq.) Coutinho, Anagallis arvensis ssp. arvensis or Ammi majus, could constitute a new source of resistance to O. ramosa for crops related weed species improvement.

References:

A parasite module was developed in the Agricultural Production Systems Simulator (APSIM) and, as an example, the module was parameterised for simulation of phasic development and biomass accumulation of *Orobanche crenata*. The root-parasitic weeds *Orobanche* spp. infect a wide variety of crops in the Mediterranean region and have already invaded the other regions with similar climatic conditions such as south Australia.

Faba bean was chosen as a host crop to study the impact of *O. crenata* infestation on its growth and yield formation. It is assumed that *Orobanche crenata* acts as an additional sink for assimilates and does not influence the host crop metabolism. The parasite carbon demand is calculated from the total number of parasite attachments and the potential growth rate of an individual *O. crenata*. The amount of dry matter partitioned into *O. crenata* is simulated based on the carbon demand of the parasite and carbon availability of the host crop by assuming that *O. crenata* has a sink priority higher than vegetative faba bean organs, but lower than the grain bearing pods.

Field experiments were conducted at ICARDA’s Tel Hadya research station in northwest Syria. Faba bean was grown both with and without *O. crenata* infestation. Simulations were run with the new developed parasite module and the modified APSIM-Faba bean module against these experimental data. Comparisons between model simulations and the measured data indicated that the APSIM-Faba bean module was able to simulate the faba bean biomass growth and yield formation. The new parasite module, together with APSIM-Faba bean, was capable of predicting the biomass accumulation of *O. crenata* and the yield loss of infected faba bean plants for various parasite infestation levels and faba bean sowing dates, as well as under different water supply conditions. Such a model can assist in the development of a systems management approach to reduce the negative impact of parasitic weeds.
17. **Striga control in maize using herbicide seed coating**

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We have developed a unique product for *Striga* control in maize. It combines low-doses of imazapyr or Pyrithiobac (systemic ALS-inhibiting herbicides) seed coating applied to imazapyr-resistant (IR) maize seed that leaves a field virtually clear of emerging *Striga* flowers season-long. This maize allows localized application of high herbicide levels on the crop seed, which are very low doses per hectare. On-station and on-farm studies over several seasons in four countries in Eastern and Southern Africa demonstrate 30 - 45 g/ha imazapyr are optimal for seed coating for effective *Striga hermonthica* and *S. asiatica* control in various environments. Low-dose herbicide seed dressing on IR-maize also controls *Striga* without impacting sensitive intercrops when they are planted 10 cm or more from maize hills. This allows small-scale farmers to continue intercropping while using maize seed treated to control *Striga*. This technology increases average yields greater than four-fold at an effective cost of less than US$4 per hectare for herbicide. The added cost is equivalent to about 25-50 kg/ha maize yield depending on market prices, suggesting potential benefit:cost ratios >25:1 even under the least favorable circumstances. This technology coupled with pulling rare *Striga* escapes can deplete the *Striga* seedbank reducing infestation of susceptible rotation crops, delay the evolution of resistant populations and be used as a stopgap until genetic crop resistance becomes available. To incorporate adaptation of IR maize to the local environment, CIMMYT initiated a breeding program originally in Harare and later in Kenya. This has focused on improving IR maize germplasm for resistance to *turcicum* blight and leaf rust diseases; increase seed of germplasm to be used for agronomic trials, on-farm research, and eventually breeder and foundation seeds for increase by seed companies; take IR maize varieties through the national performance trials in Kenya; and convert elite lines to herbicide resistance and use them to develop adapted IR-maize hybrids for the region. High yielding and disease resistant IR-maize inbred lines, hybrids, and open pollinated varieties adapted in *Striga* infested agro-ecologies in sub-Saharan Africa will soon be available from CIMMYT. The goal is to deliver this product to farmers in all major agro-ecologies in sub-Saharan Africa where *Striga* is endemic by cooperating with herbicide producers and seed companies.

Funding from the Rockefeller Foundation

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\(^1\)Kanampiu, F. K., Ransom, J. K. and Gressel, J. (2001) 'Imazapyr seed dressings for *Striga* control on acetolactate synthase target-site resistant maize', *Crop Protection*, 20, 885-895.  
\(^2\)Kanampiu, F. K., Ransom, J. K., Friesen, D. and Gressel, J. (2002) 'Imazapyr and pyrithiobac movement in soil and from maize seed coats controls *Striga* in legume intercropping', *Crop Protection*, (in press),  
18. Potential use of systemic acquired resistance for broomrape control

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It has been shown in different plant systems that typical defence reactions against phytopathogens are also induced in response to parasitic plant infection (i.e. PRs, phenolics). The induction of such responses could be very effective in preventing parasitism by blocking early stages of the biological cycle of the parasite. If this is the case, we can hypothesize that activation of defense reactions can be developed as broomrape control strategy and it can be done by exploiting the so-called “Systemic Adquire Resistance” (SAR) phenomenom.

A number of chemicals including the natural salicylic acid and their functional analogues INA and BTH, among other natural and synthetic compounds, induce SAR in a number of either monocot and dicot plants. Work carried out in our group have revealed that these compounds have been effective in controlling some sunflower pathogens (i.e. Puccinia helianthi, Plasmopara halstedii, Botritys cinerea), but no others (i.e. Sclerotinia sclerotiorum) (Gómez-Rodríguez MV et al., 1999. Resistencia sistémica adquirida en girasol (Helianthus annuus L.). XIII Reunión de la Sociedad Española de Fisiología Vegetal. Sevilla, Spain; Prats et al., 2002. Acibenzolar-S-methyl-induced resistance to sunflower rust (Puccinia helianthi) is associated with an enhancement of coumarins on foliar surface. Physiological and Molecular Plant Pathology. In press.; Prats et al., 2001. Inducción La utilización de inductores de resistencia sistémica adquirida en el control de plantas parásitas. In: Uso de herbicidas en la agricultura del siglo XXI (De Prado R, Jorrín JV, eds.). Servicio de Publicaciones de la Universidad de Córdoba; other unpublished results).

In the last two years we have evaluated by either field and greenhouse experiments the effect of SAR activators on angiosperm parasitism by mainly using the sunflower-O. cernua interaction as a model system. Data and main conclusions will be presented during this meeting. Results on a similar work have been recently published (Saueborn et al., 2002. Phytopathology 92: 59-64).

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19. Search for specific targets in *Orobanche* for chemical control


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That the methods actually applied to manage broomrape are not efficient and specific is unfortunately seen by many farmers and researchers. One of the activities of our reasearch team is to characterize nitrogen and carbon metabolism of *Orobanche* and *Striga*, hoping primarily to identify some metabolic process that play a major role in the parasitic way of life of these species and secondly to identify some specific inhibitors usable as selective herbicides. Our taking part in the Joint Meeting in Obermarchtal on the Parasitic Management in Sustainable Agriculture will be then the opportunity to present a statement of our activities, including the most recent data obtained in our laboratory.

Paradoxically, despite the fact that sucrose conversion into mannitol followed by a great accumulation of this compound is well-known to be specific and likely essential to *Orobanche* and *Striga* as compared to their typically sucrose-producing hosts, few studies are developed to characterize inhibitors of these process. The gene and the enzyme (Mannose 6-phosphate reductase, M6PR) essential to mannitol synthesis in broomrape and *Striga* are now characterized (1, 2). Since the works of Robert et al. published in 1999 on the characterization of the molecules acting *in vitro* as competitive inhibitors on *Orobanche* and *Striga* M6PR (3), many others compounds were tested *in vitro* and identified as more potent inhibitors (4). Their activity *in planta* is analyzing on *Striga* cals and plants fixed to *Sorghum*. The first results are promising : the impact on the infested plant is low and the parasite death is observed few days following the treatment. Nevertheless, additonal works are necessary to characterize clearly the metabolic changes involved in the parasite death.

*Striga* exhibits after emergence a high and uncontrolled transpiration rate resulting when nitrogen fertilization (nitrate) is applied on cultures in an intensive and not-regulated flux of nitrate and glutamine from the roots of the infested host towards the parasite leaves (5). In contradiction with some previous works that reported a low ability of *striga* to assimilate nitrate, the parasite exhibits an effective strategy of detoxification of N-excess through the incorporation of the host-derived nitrogen into a non-toxic compound, asparagine. Indeed, the activity of asparagine synthetase, the enzyme essential to Asn synthesis in plants, has been recently detected in *Striga* and the gene sh-AS was also characterized (6). Specific inhibitors of AS are known and effects of these compounds on *Striga* development will be tested soon.

4. Rousset A., 2002, Ph.D, data not published
6. Pouvreau J.B., Master, data not published
20. Assessing relative efficacy of Nijmegen 1 for Striga control

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Striga hermonthica parasitizes a number of economically important crops. It affects two-thirds of the 73 million hectares of land under cereal production in the sahelian and savannah zones of Africa where it threatens the livelihood of over 100 million people. The infested area continues to increase because intensification of land use (D. Chikoye, personal communication). Various in season control measures are suggested to combat the weeds. One option is to develop herbicide resistant crops (e.g., maize) that seem to give good control. Currently it is not an option for the resource poor farmers. Common herbicide options cannot usually control Striga before or immediately after attachment and thus prevent damage of the crop.

GR24 and Nijmegen 1 are germination stimulants, whose ED$_{50}$ values are in the order of 10$^{-9}$ and 10$^{-6}$ mol L$^{-1}$. GR24, the most potent one, has two stereogenic centres so mixtures of diastereomers are obtained during the synthesis. Nijmegen 1 contains only one chiral centre and may be attractive for large-scale preparation.

Nijmegen 1 has been tested under field conditions to control Orobanche. Soil seed bank depletion was 75% at 0-10 cm depth and dropped to about 35% at 10-20 cm depth.

Proper research and development of Nijmegen 1 require in depth knowledge of its behaviour in the soil such as its adsorption, mobility and fate and how to spray the compound with a manageable carrier volume. Proper formulations of the stimulant could perhaps increase efficacy and distribution in the soil.

It will require well-structured protocols in controlled environment to unravel the behaviour of Nijmegen 1 in the soil and relate it to efficacy, e.g. using contrasting soils types (clay and organic matter content, soil acidity and CEC) and moisture regimes in conjunction with studies of the fate of the compound in the rhizosphere. These studies may help understanding the relative efficacy under contrasting soil and environmental conditions.

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4 Miele S, Benvenuti, S, Macchia M & Pompeiano A 2001. Orobanche control in tobacco by using a seed bank germination stimulant. Combined meeting of Working Groups 1, 2, 3 and 4 of COST Action 849, Parasitic Plant Management in sustainable Agriculture, 10-20 October 2001 Bari Italy, 28
The application of germination stimulants for *Orobanche* control by the induction of suicide germination appears attractive for several reasons. It is highly specific for *Orobanche* (and other parasitic plant) seeds. Ecological side-effects are not expected nor have they been observed. Due to their high biological activity they are applied in very low amounts and they can be applied on blank soil before planting the crop. As they are decomposed in the soil within short time, uptake and accumulation in the crop plants is excluded.

The application of germination stimulants of course requires to obey the conditions for *Orobanche* germination: wet conditioning prior to germination stimulation, after 8-10 days application of the chemical, appropriate (optimal) concentration of the chemical in the soil, leaving the soil without host plants for a period of 8-10 days. The elimination of the whole *Orobanche* seedbank during a single treatment cannot be expected. However, after several treatments during a sequence years the *Orobanche* seedbank will be reduced to a minimum.

The application of germination stimulants is a biological control measure for *Orobanche*. Since natural strigolactones cannot be isolated for commercial application due to their extremely low concentrations, Binne Zwanenburg and I are exploring the application of synthetic structural analogues of the strigolactones for field application. Experiments with Nijmegen-I, synthesized by Binne Zwanenburg’s group, which I got formulated for field application by the BASF AG, were carried out in several institutions worldwide with very promising results. These results will be presented.

The present problem is the official registration and release of germination stimulants for field application in agricultural fields. Germination stimulants are not herbicides or pesticides, but they are considered as agrochemicals. The foundation of a consortium of interested institutions could be very helpful for further exploration of this smooth way of *Orobanche* control.
22. Management of *Orobanche* spp. in vegetable 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 underway. In broad beans, *Orobanche* was effectively controlled by two sprays of glyphosate, at 45 to 90 g a.i. per hectare, the first applied at the beginning of broad bean flowering and the second 14 days later. In cabbage, broomrape was effectively controlled in the field by spraying twice with glyphosate at 60 to 100 g a.i./ha or imazaquin at 5 to 10 g a.i./ha. In celery, two applications of glyphosate at 40 to 50 g a.i./ha controlled broomrape and allowed the celery heads to attain full size, while in tomato and eggplant broomrape was completely controlled in the field by mulching the soil with black polyethylene sheeting on the day of transplanting. Glyphosate and sulfosate, applied twice at 30 to 50 g a.i./ha, were also very effective against broomrape, but reduced yield of tomato. Despite severe toxicity to eggplants, yield was not reduced. Imazaquin applied twice, at 5 to 10 g a.i./ha, was not very effective. Rimsulfuron, applied at 10 to 20 g a.i./ha, reduced broomrape dry weight and number of shoots. It was safe on tomato but was toxic to eggplant fruits causing malformation and splitting. Surface application of charcoal (15 g/m²) to the soil had no effect on broomrape infestation, whereas trifluralin incorporation at 900 g a.i./ha prior to planting had insignificant effect. In greenhouse-grown tomato, soil solarization for 3 and 6 weeks controlled broomrape by 75% and 99%, respectively. 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. Results obtained so far show that covering the soil with black polyethylene gave in all cases the best results, significantly reducing *Orobanche* infestation and increasing yield.
Orobanche ramosa and mostly the sunflower broomrape (Orobanche cumana Wallr.) are major pests in sunflower and tobacco fields in Romania. Infestation may reach levels that prohibit further planting of sensitive crops.

In Romania the most severe losses from broomrape, in sunflower fields, have been recorded in southern Moldavia, eastern Muntenia and Dobroudja, where the cultivars cropped, formerly known as resistant, have been progressively attacked by this parasite.

Thus, research on parasite – host plant relationships have been amplified, leading to identification of differential set of sunflower genotypes, as well as to obtaining the resistant sunflower hybrids, wholly to the attack of all broomrape races occurring in this country. With the development of resistant varieties, more aggressive races of Orobanche cumana are developing. For this reason, for breeding, wild sunflower species provided sources of resistance to this parasite.

Other control measures or integrated plant management schemes need implemented. Ever since, some of research was conducted trying to control broomrape with herbicides. The herbicides used in the experiments showed some broomrape control but some of them had a pytotoxic effect on sunflower.

Breeding tobacco for broomrape resistant is on enough difficult goal. In order to diminish the negative effect of broomrape on tobacco yield, it were tested different methods: crop rotation, deep ploughing, biological control, by trap plants, insects, fungi or resistant forms germination stimulators, herbicides.
24. *Cuscuta* Tolerates High Rates of Herbicides Inhibiting Amino Acid Biosynthesis

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The effect of amino acid biosynthesis inhibitors (AABI), including EPSPS and ALS inhibitors, on *Cuscuta* seedlings was studied in a sand-Petri dish bioassay. The germination and shoot elongation of scarified seeds exposed to the herbicide was measured 4 days after sowing (DAS). The validity of the bioassay was confirmed by comparing the response of *Cuscuta* seedling to the response of other susceptible and resistant species and by exposing *Cuscuta* seeds to trifluralin, a microtubule assembly inhibitor that effectively controls the parasite. Sulfoemuron concentrations above 0.1mM were needed to inhibit *Cuscuta* shoot development by 50% (I50), while at 0.01µM the herbicide inhibited sorghum root development completely. High concentrations of glyphosate (I50 = 52mM) were needed to inhibit *Cuscuta* shoot growth, whereas the I50 for RR cotton root elongation inhibition was 6.2mM, and a I50 value of 0.08mM was found for both, susceptible cotton and Sorghum. *Cuscuta* exhibited a unique and high tolerance to several other AABI tested, while remaining sensitive to trifluralin (1µM). Shikimic acid accumulation in developing seedlings was used as an indication for glyphosate inhibition of the EPSPS. In *Cuscuta* seedlings an increase in shikimic acid was detected before any inhibition in shoot elongation was visible, hence confirming that the herbicide had penetrated the seeds and that the parasite has an active EPSPS.

Glyphosate and sulfoemuron were applied post-emergence on sulfoemuron-resistant transgenic tomato (micro tomato) and transgenic glyphosate-resistant sugar beet (RR) infested with *Cuscuta*. Three days after herbicide application parasite growth was inhibited. However, 4 weeks after application 50% of the *Cuscuta* plants resumed growth, set seeds, and caused severe damage to the host plants. The herbicide application to *Cuscuta*-infested hosts had very little impact on improving host development as indicated by the small increase in the sugar beet fresh weight or tomato yield.

The response of *Cuscuta* seeds and the recovery of the parasite on AABI herbicide-treated host plants raise a number of questions regarding the biochemical mechanism(s) involved in the parasite, the nature of the host-parasite association and the feasibility of using herbicide-resistant hosts as an effective means for *Cuscuta* control.
Host specificity is a distinctive feature of parasitic plants from genus *Orobanche*. It has been widely demonstrated that broomrape seeds germinate when exposed to stimulants excreted from host plant roots. It allows them to remain in the soil more than twenty years and germinate when the exact host plant is grown in the field. In addition to this more or less narrow host specificity, the parasite seeds are able to respond to different synthetic germination stimulants, as those from GR family. These stimulants provoke the germination of parasite seeds from different *Orobanche* species, avoiding somehow parasite host specificity.

Up to now very little is known about the mechanism of this specific germination. It is known that plant hormones change during the germination in the seeds of any plant species. Gibberellic acid (GA) is produced in the embryo and carried in the endosperm during the imbibition process of barley seeds. Brassinosteroids and gibberellins promote tobacco and *Arabidopsis* seed germination.

There are some plant hormones that were found to take place in *Orobanche*-host interactions. Gibberellins were identified from both *Orobanche minor* and its host *Trifolium repens*. The parasite has the ability to produce gibberellins as GA$_{38}$, GA$_{47}$ and GA$_{58}$. Brassinosteroids shortened the conditioning period required for germination of clover broomrape seeds and increased the rate of seed germination induced by host root exudates. GA$_3$ has also a positive effect on conditioning and seed germination of clover broomrape (*O. minor*). In our previous experiments exogenous added IAA and GA$_3$ were found to provoke germination of *Orobanche ramosa* seeds. The treated seeds produced callus and germination like structures. Under the experimental conditions ABA inhibited parasite seeds germination, when it was elicited by cotylenins (metabolites of fungus *Cladosporium* spp.). These made us to propose that probably plant hormones play an important role in the mechanism by which the host stimulants trigger the germination or parasite seeds.

We investigated the IAA and ABA synthesis during the seed germination of two *Orobanche* species, *O. ramosa* (infesting tobacco) and *O. cumana* (infesting sunflower). Seeds of both species were exposed on the root exudates from host and non-host plant. Control seeds in water were always maintained. IAA was released from *Orobanche* seeds during the germination in host parasite interactions, comparing to non-host-parasite interactions. Moreover IAA was released by the germinating seeds as early as 24 hrs after seeds of *O. ramosa* were exposed to the germination stimulant (host root exudates). The quantity of IAA released was not corresponding to the final number of germinating seeds. ABA remained unchanged during the germination of the parasite seeds.

The result show that IAA production is not a consequence of the germination process, but rather a step of the mechanism, triggering the germination upon the induction by the host factor and finalised with germinating seeds.
26. Control of Orobanche crenata in horticultural faba beans of determinate habit.

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Broomrape is a major constraint for legume cultivation in large areas of the Mediterranean and West Asia. Several control strategies have been employed such as delayed sowings, long rotations, trap and catch crops, hand weeding, solarisation, herbicides, biological control and genetic resistance, but all without unequivocal success and over indeterminate habit plants. The control over indeterminate growth plants have been achieved with glyphosate at low concentration in a double application (60 g/ha) when applied at the right stage, the first treatment applied at early establishment of broomrape, before emergence, and a second one two weeks later. Higher concentrations induce chlorosis and rolling of apical leaves and slow growth in the standard indeterminate growth habit cultivars, but might be better tolerated by determinate growth habit cultivars.

Glyphosate effectively controls broomrapes at standard rates (67 g a I/ha), with improved control at increasing rates (201 g a i/ha) being total at 336 g a i/ha. However, despite this reduction in the broomrape infection, green pod yield did no increase significantly from rate 0 to rate 3 (201 g a i/ha), what could be explained by an escape to broomrape damage due to the early harvest.

The main benefit of a faba bean cultivar for green pod consumption of determinate growth habit is the feasibility of a single early harvest that can even be mechanised. This can be profitable for the farmer as green pods and seeds for fresh consumption are very appreciated by the Spanish market. In standard sowing dates by November, this early harvest is made at early stages of broomrape establishment, when we can expect little yield losses, with the important added value of a reduction of the broomrape seed-bank in the field, thus being a profitable trap crop.

Retaca can be considered a catch crop, that induce germination of the broomrape seeds and allow its establishment. Hower, it is harvested for green pod before the broomrape emerge and reproduce, thus reducing the parasitic weed seed bank in the soil. This would represent a catch crop not only beneficial in the long term reducing future infections, but also profitable at the short term for farmers as the young green pods are very appreciated in the local market.

An added value of determinate cultivars for young green pod consumption is that the early harvest would represent an escape not only to the crenate broomrape (O. crenata) but also to other broomrape species such as O. foetida that cause severe damage in faba bean fields in areas of Tunisia.
27. Need to integrate several control methods to solve the broomrape (*Orobanche crenata*) problem in pea in Southern Spain

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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, but there is a strong interest to increase pea cultivation in the area due to its high potential in winter sowings. However, as soon as acreage increased, broomrape (*O. crenata*) emerged as a major constraint. All the cultivars available to farmers are extremely susceptible. We detected sources of resistance in accessions of *P. sativum*, *P. abyssinicum*, *P. arvense*, *P. elatius* and *P. fulvum* (Rubiales *et al.*, 1998) 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. Resistance ought to be integrated with other control methods to ensure an adequate protection and to ensure its durability.

Glyphosate has proven effective in controlling *O. crenata* in faba beans, but not in peas that are very sensitive. However, tolerance to glyphosate exists in pea germplams that could be exploited in breeding (Sillero *et al.* 2001). Further, pea could be genetically engineered with the glyphosate-resistance gene. New herbicides, such as imazethapyr and imazapyr can be applied as pre-emergence treatments or as seed dressing for broomrape control (García-Torres *et al.*, 1998; Jacobsohn *et al.*, 1998). However, these treatments should be complemented with specific post-emergence treatments when the conditions are very conducive to infection, and have not yet been adopted by farmers.

Foliar applications of BTH (activator of systemic acquired resistance) provided a 20-30% of reduction of broomrape infection under field conditions. However the technology needs to be improved before it can be recommended to farmers.

Because of the extreme difficulty of controlling broomrape, prevention is of great importance. On a local level, the sources of infection can be reduced by controlling the use of contaminated seed lots, or simply by destroying heavily infested fields.


28. Broomrape Control in tomato and sunflower

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Egyptian broomrape (*Orobanche aegyptiaca*) is a devastating pest in the processing tomato fields in Israel. Sulfosulfuron is a sulfonylurea herbicide registered for weed control in wheat in many courtiers around the world. The herbicide was found to be highly selective to tomato and was tested in the last two years for his efficacy to control broomrape in tomato. Based on the results we obtained, the herbicide was registered for broomrape control in tomato on a limited area of 50 ha for one year for evaluation purposes. The main obstacle of the present control method is the sprinkler irrigation system, which must be left in the field for one month after planting. This year we tried to find solutions to overcome this obstacle and to fine-tune the application rates and to adopt it to the various infestation levels. The results obtained in these experiments will be discussed.

Sunflower broomrape (*Orobanche cumana*) cause severe yield and quality losses in Israel each year. Infested fields are being abundant and the farmers avoid growing sunflower in these fields. In the recent years resistant varieties were developed and rapidly spread on most of the sunflower growing areas. All the resistant varieties driven from one resistant source is temperature dependent being sensitive at lower temperatures. Sunflower planting dates are February - March and the farmers tend to sow as early as possible to save precious water. This caused a broomrape outbreak even with the resistant varieties because of the low temperatures prevailing in February – April. In order to protect the resistant verities from Broomrape attack at the cold period we applied imazapic when the first broomrape attachments appeared on the roots. The results we obtained will also be discussed.
Three main chemical approaches are used in agriculture for the control of *Orobanche*: soil fumigation, foliar and soil application of herbicides.

In soil fumigation methyl bromide is mainly used, but metam (metham-sodium), dazomet and 1,3-dicloropropene are also applied. Glyphosate, several sulfonylureas and imidazolinones have been evaluated as foliar applications against *Orobanche* spp. in various crops. The efficacy of some of them applied in the soil has also been studied.

In order to evaluate the potential consequences on the environment of the chemicals used against *Orobanche*, information is presented below related mainly to their fate in soil or water.

**Methyl bromide** (fungicide, nematicide, herbicide and insecticide): Extremely phytotoxic. Important residues of this chemical and of bromide ions in fumigated soils are reported in the literature.

**Metam or metham-sodium** (fungicide, nematicide, herbicide and insecticide): Highly phytotoxic. In soil, it is rapidly decomposed to methyl isothiocyanate, which is volatile. Dissipation time (DT$_{50}$) ranges from 23 min to 4 d.

**Dazomet** (nematicide, fungicide, herbicide and insecticide): Highly phytotoxic. In soil, it is rapidly decomposed to methyl isothiocyanate, which is volatile. Dissipation time (DT$_{50}$) ranges from 23 min to 4 d.

**1,3-dicloropropene** (nematicide): Phytotoxic, non-persistent in soil, undergoing hydrolysis to the corresponding 3-chloroallyl alcohols. DT$_{50}$ ranges from 2.4 d to 17 d.

**Glyphosate** (non-selective herbicide): Strongly adsorbed to the soil and becomes immobile. Microbial degradation is the major cause of loss from soil, with liberation of carbon dioxide. The principal metabolite is aminomethylphosphonic acid. DT$_{50}$ ranges from 3 d (trimecium salt) to 60 d (acid).

**Imazapic** (herbicide): Based on models, the estimated environmental concentrations (for chronic exposure) are 1.5 ppb for surface water and 14 ppb for ground water.

**Imazethapyr** (herbicide): Its half-life in soil is 1-3 months.

**Imazaquin** (herbicide): It is steadily degraded in soil by microbial activity and photolysis. It may remain active in the soil for several weeks to several months, depending on environmental conditions.

**Sulfosulfuron** (herbicide): The estimated environmental concentrations for surface and ground water are 1.7 ppb and 0.3 ppb respectively.

**Rimsulfuron** (herbicide): It is degraded rapidly in soil, predominantly via chemical pathways. Microbial degradation plays a minor role. Rates of degradation are influenced by pH (most stable in neutral pH soil, degrades more rapidly in alkaline and acidic soils). DT$_{50}$ ranges from 1.7 d to 4.3 d.

**Chlorsulfuron** (herbicide): In soil, deactivation is through hydrolysis followed by complete metabolism to low-molecular-weight compounds through normal soil microbial processes. Rate of hydrolysis is increased by higher soil temperature, low pH and the presence of moisture. Average half-life under growing-season conditions is 4-6 weeks.

**Triasulfuron** (herbicide): The degradation behaviour in soil is determined by the soil type, pH and especially temperature and moisture content. Field studies with silty loam, clay loam and sandy loam showed DT$_{50}$ from 12 d to 45 d.
30. *Synthesis of Sesquiterpene Lactone Modes as Orobanche cumana seed germination Elicitors*

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Broomrapes are common parasitic weeds of the genus *Orobanche* affecting major dicotyledoneous crops, such as legumes, tomato, tobacco, and sunflower. The lifecycle of these parasites is closely attached to their hosts, since they need a specific chemical signal exuded by to roots to serve as a germination inductor.1 Up to date, a whole family of host germination stimulants for *Striga* spp, another problematic parasitic weed, and *Orobanche* spp. named strigolactones has been characterized2 At the same time, a whole collection of strigolactones analogs named GR-family have been developed and tested aiming for larger chemical stability and easy multigram-scaling synthesis.3 Several approaches have received attention to control these weeds. Hand-weeding methods can be discarded since the damage is already done in the crop roots. The use of crop rotation with non-hosts crops that do induce suicidal germination is another possibility. However, the use of these crops may be of no economical interest under many circumstances. Several herbicides have been tried for parasitic weed control with promising but without fully satisfactory results.4 The “suicidal germination” approach is another method of control that is receiving attention and constitutes the basic clue for the development of the GR family of synthetic inductors. Besides sorgolactones, several sesquiterpene lactones (SL) have been tested as potential inductors of the germination of *Striga* spp.3 Some fungal metabolites have also been tested on *Striga* and *Orobanche*,6 with promising results.

Following the last mentioned approach, we successfully tested several SL with guaianolide, *trans*,*trans*-germacranolide, eudesmanolide and melampolide backbones as possible inductors of *Orobanche cumana*, *O. crenata*, *O. ramosa*, and *O. aegyptiaca* seed germination. Those lactones bearing features common to many SL isolated from sunflower were able to induce germination of the specific sunflower parasite *Orobanche cumana* at lower concentrations than the positive control GR 24. Moreover, active compounds did not exert any inducing germination activity on the rest of *Orobanche* species tested. They have been selected aiming to test two different hypothesis: i) how important is the presence of Michael acceptors since such a mechanism has been proposed as responsible for the induction of the germinations for strigolactons;3a and ii) how much influence does the type of backbone exert. Comparison between common chemical features and conformations of both GR-24 and SLs are discussed.

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In the past decade biotechnological methods for the improvement of transgenic plants have been elaborated and plants with novel recombinant genes were tested in different agricultural environments. A few transgenic approaches were quite successful on economical and even on ecological bases. Advanced technologies like genomics and proteomics enhanced the possibilities for understanding of complex plant interactions. Differential expression methods enable us to identify key genes involved in either as plant pathogen or parasite interactions.

Genetic engineering is the interface between the descriptive methods and the applied plant biotechnology. In the future these technologies will serve more and more the plant breeding activities in the major bottlenecks of resistance improvement like parasitic weeds. The genetic transformation of some pulse crops, namely, chickpea and lentil is still in their infancy compared to model plants. Nevertheless reliable transformation methods for pea and field bean have been elaborated in some laboratories in Europe. The LG-Molekulargenetik at the University of Hannover has been developed reliable transformation protocols for pea and field bean which can be used for control of parasitic weeds mainly in the Mediterranean region and also in Africa where the above disease is severe. In genetic engineering, the traditional interference strategies are the over expression or the silencing of genes under the control of constitutive promoters. On the basis of the new possibilities for the identification of key genes and on the basis of intensive biochemical and functional characterisation of plant defence substances, the genetic engineering approaches will become more predictable. In addition to the traditional methods the elucidation of gene silencing mechanisms have recently opened up a new avenue for weed control e.g. Orobanche and Striga. With an expectation that the gene silencing signals are transmitted in the plant as a kind of systemic reaction may be considered as a tool for targeted control of the weeds by genetically engineered host plants. Provided that the gene silencing signal will pass the haustorium and that weeds are also susceptible for induced gene silencing. It is very important to know which gene/s are responsible for the weed to become parasitic to the host plant. As soon as it is known at the molecular levels, the RNAi can be constructed for weed specific genes which may be expressed under the control of either organ specific or on inducible promoters.