

Joint Working Groups and MC meeting of COST Action 849,  
*Broomrape biology, control and management* 15-17 September 2005, Reading University, UK.



The University of Reading



Department of Agriculture, School of Agriculture, Policy  
and Development

## ABSTRACTS

### COST Action 849: Parasitic Plant Management in Sustainable Agriculture

Published September 2005

The University of Reading, Department of Agriculture.

COST Action 849

Website: <http://cost849.ba.cnr.it/>



# Broomrape biology, control and management

Joint Working Groups  
Workshop  
The University of Reading  
15-17 September 2005

# CONTENTS

## **WG1: Broomrape germination biology 3**

**Biosynthesis of germination stimulants of parasitic plants and their biological function (Bouwmeester) 5**

**The biosynthetic origin of strigolactone germination stimulants of the plant-parasitic *Striga* and *Orobanch*e spp (Matúšová) 6**

**Inhibitors and stimulators of germination of *Orobanch*e (Mayer) 7**

**Isolation and characterization of genes involved in the formation of the germination stimulants for the parasitic weed, *Striga* (Zhongkui Sun) 8**

**Influence of fluridone on conditioning and germination of *Orobanch*e seeds (Joel) 9**

**Modelling seed dormancy and germination (Murdoch) 10**

**Processes and rates of *Striga hermonthica* seed bank depletion as a result of fallow and different crop covers (Van Mourik) 110**

## **WG3: Resistance Breeding 11**

**Involvement of protein cross-linking, peroxidase and  $\beta$ -1,3-endoglucanase in resistance of pea against *Orobanch*e *crenata* (Pérez-de-Luque) 13**

**Search for a scheme of host responses to *Orobanch*e (Thoirin) 15**

**Tomato/*O. ramosa* interaction: pathogen perception and defence elicitation (Lejeune) 16**

**Sunflower genotypes resistant to the most virulent populations of broomrape in Romania (Păcureanu) 17**

**Resistance to new virulent *O. cumana* races (Fernández-Martínez) 19**

**Mapping of quantitative trait loci for resistance to *Orobanch*e *crenata* in pea (Fondevilla) 19**

## **WG1: Taxonomy 221**

**Genetic variation in *Orobanch*e *foetida* as revealed by AFLP analysis (Vaz Patto) 21**

**Preliminary results on genetic analysis of Greek *Orobanch*e populations using RAPDs (Lyra) 22**

**Taxonomic changes in *Orobanch*e and related genera (Rumsey) 23**

**WG2: Biological control 24**

Transforming *NEP1* toxin gene and other genes into two *Fusarium* spp. to enhance mycoherbicide activity against *Orobanchae* – failure, success and progress (Gressel) 24

Compatibility of irrigation systems with the application of broomrape biocontrol agents (Vurro) 26

Use of fungal metabolites for broomrape suicidal germination (Vurro) 27

Natural enemies of *Orobanchae* species in Slovakia (Tóth) 28

Biocontrol agents for *Orobanchae* - A seaweed product: a new potential germination stimulant for *Orobanchae ramosa* (Economou) 30

**WG4: Integrated control and biodiversity conservation 33**

*Orobanchae crenata* control on legumes in various intercrops (Rubiales) 33

Use of herbicide resistant crops in Greece for control of *Orobanchae* and other weeds (Kotoula-Syka) 35

New advances in chemical control of *Orobanchae aegyptiaca* in tomato (Lande) 37

Lessons learned from integrated control of *Orobanchae* in Cyprus (Vouzounis) 38

Control of *Orobanchae* on sunflower and tobacco crops in România (Jinga) 40

Minirhizotron- a new method for in-situ modeling of the underground development of *Orobanchae* (Eizenberg) 41

*Rhinanthus minor* (Yellow rattle) – Grassland weed or the ecologist's friend? (Westbury) 42

**Climate change 43**

Predicting the impacts of climate change on crops (Wheeler) 44

## WG1: Broomrape germination biology

### **Biosynthesis of germination stimulants of parasitic plants and their biological function (Bouwmeester)**

**Harro Bouwmeester**, Kumkum Rani, Esther van Echteld and Radoslava Matusova  
*Plant Research International, P.O. Box 16, 6700 AA Wageningen, The Netherlands*  
E-mail address of presenting author: [harro.bouwmeester@wur.nl](mailto:harro.bouwmeester@wur.nl)

Parasitic plants of the *Striga* (witchweeds) and *Orobanche* (broomrapes) genera can only survive on the roots of a host plant and must obtain most of their resources from them. Seeds of parasitic plants are tiny, and after germination they must attach to a host root within days or otherwise they will die. Parasitic plants have evolved a graceful strategy to deal with this requirement: their germination depends unconditionally on compounds that are produced by the roots of their hosts in extremely low concentrations. Clearly, the chemical signaling involved in this first step in the lifecycle is crucial in the life of these parasitic plants (Bouwmeester *et al.*, 2003). Therefore, several research groups have studied the chemistry of this interaction and for both parasitic plant genera and a range of hosts several of the germination stimulants were identified (Cook *et al.*, 1972; Hauck *et al.*, 1992; Siame *et al.*, 1993; Butler, 1995; Yoneyama *et al.*, 2001; Yoneyama *et al.*, 2004). Perhaps surprisingly, considering the wide range of host species from very distinct plant families, all the germination stimulants identified so far belong to one chemical class of compounds, the strigolactones. The strigolactones were identified in the past as sesquiterpene lactones and this identification was taken over by all following publications. The correct identification of the biosynthetic origin is of great importance if we want to understand the biosynthetic regulation. Recently we have elucidated the biosynthetic origin of the germination stimulants of *Striga hermonthica* and *Orobanche crenata* in maize, sorghum and cowpea (presentation by Radoslava Matusova) (Matusova *et al.*, 2005). Considering the presence of strigolactones in the mono- and dicotyledonous species mentioned above and in a range of other unrelated plant species (cotton, red clover, tomato, *Lotus japonica*, *Menispermum dauricum*), we postulate that the strigolactones play an essential role in plant biology and are therefore highly conserved in the plant kingdom.

Until recently, the significance of these signalling compounds for the plant itself has remained elusive (why do plants produce strigolactones when they are obviously disadvantageous, since they cause parasitism). The fact that they have persisted despite the supposedly strong counter-selection suggests that they are important. Indeed, an intriguing recent paper in *Nature* has shown that the strigolactones are used by arbuscular mycorrhizal fungi for their colonisation process (the strigolactones are the branching factor that is required for mycorrhizal mycelia to become infective) and this most likely answers the question why plants still produce strigolactones (Akiyama *et al.*, 2005). Many plant species including the majority of plants that are hosts to *Orobanche* and *Striga* spp can host arbuscular mycorrhizae, a beneficial relationship between plant roots and certain root-inhabiting fungi

(Johansson *et al.*, 2004). We will discuss whether the interaction between mycorrhiza and parasitic plants is mediated via the exchange of information via the host plant.

## References

- Akiyama K, Matsuzaki K, Hayashi H** (2005) Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature* **435**: 824-827
- Bouwmeester HJ, Matusova R, Zhongkui S, Beale MH** (2003) Secondary metabolite signalling in host-parasitic plant interactions. *Current Opinion in Plant Biology* **6**: 358-364
- Butler LG** (1995) Chemical communication between the parasitic weed *Striga* and its crop host. A new dimension in allelochemistry. *In* K Inderjit, FA Einhellig eds, *Insights into Allelopathy*, Vol ACS Symposium Series. ACS Books, Washington, pp 158-168
- Cook CE, Whichard LP, Wall ME, Egley GH, Coggon P, Luhan PA, McPhail AT** (1972) Germination stimulants. 2. The structure of strigol-a potent seed germination stimulant for witchweed (*Striga lutea* Lour.). *Journal of the American Chemical Society* **94**: 6198-6199
- Hauck C, Muller S, Schildknecht H** (1992) A germination stimulant for parasitic flowering plants from *Sorghum bicolor*, a genuine host plant. *Journal of Plant Physiology* **139**: 474-478
- Johansson JF, Paul LR, Finlay RD** (2004) Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. *FEMS Microbiology Ecology* **48**: 1-13
- Matusova R, Rani K, Verstappen FWA, Franssen MCR, Beale MH, Bouwmeester HJ** (2005) The strigolactone germination stimulants of the plant-parasitic *Striga* and *Orobanch* spp are derived from the carotenoid pathway. *Plant Physiology*, in press
- Siame BA, Weerasuriya Y, Wood K, Ejeta G, Butler LG** (1993) Isolation of strigol, a germination stimulant for *Striga asiatica*, from host plants. *J. Agric. Food Chem.* **41**: 1486-1491
- Yoneyama K, Takeuchi Y, Sato D, Sekimoto H, Yokoka T** (2004) Determination and quantification of strigolactones. *In*: DM Joel ed 8th International Parasitic Weed Symposium. The International Parasitic Plant Society, Durban, South Africa, pp 9
- Yoneyama K, Takeuchi Y, Yokota T** (2001) Production of clover broomrape seed germination stimulants by red clover root requires nitrate but is inhibited by phosphate and ammonium. *Physiologia Plantarum* **112**: 25-30

## **The biosynthetic origin of strigolactone germination stimulants of the plant-parasitic *Striga* and *Orobanch*e spp (Matúšová)**

**Radoslava Matúšová**<sup>1</sup> and Harro J. Bouwmeester<sup>2</sup>.

<sup>1</sup>*Institute of Plant Genetics and Biotechnology, Slovak Academy of Sciences, Akademická 2,  
P.O.Box 39A, 950 07 Nitra, Slovakia*

<sup>2</sup>*Plant Research International, WUR, P.O. Box 16, 6700 AA Wageningen, The Netherlands*

*Email address of presenting author: [nrgmatu@savba.sk](mailto:nrgmatu@savba.sk)*

The seeds of parasitic plants of the genera *Striga* and *Orobanch*e will only germinate after induction by a chemical signal exuded from the roots of their host. For both *Orobanch*e and *Striga* spp. several germination stimulants were identified from host and non-host plants. Most of these compounds were shown to be isoprenoid and belong to one chemical class, called the strigolactones. The germination stimulants have been grouped as sesquiterpene lactones. Although their structures have been known for several decades, little is known about how these compounds are produced by the host plants.

Isoprenoids are biosynthesized via two independent pathways: the cytosolic mevalonic acid (MVA) pathway and the plastidic methylerythritol phosphate (MEP) pathway. Monoterpenes, diterpenes, carotenoids, the plant hormones abscisic acid and gibberellins, the side chains of chlorophylls, plastoquinones and phylloquinones are biosynthesized via the plastidic MEP pathway. Sesquiterpenes, sterols and triterpenes are produced from the cytosolic MVA pathway. We focus on the biosynthesis of the strigolactone germination stimulants for *Orobanch*e and *Striga* spp. in several host and non-host species (e.g. maize, sorghum, cowpea): Two different approaches to unravel the biosynthetic pathway of germination stimulant(s) were used;

- 1) the specific inhibitors of isoprenoid pathways were analysed for altered germination stimulant production on single seedlings by induced/reduced *Striga hermonthica* and *Orobanch*e *crenata* germination;
- 2) the characterised mutants in the predicted biosynthetic pathway were analysed as well.

We will discuss the progress of our research into the biosynthetic origin of strigolactone germination stimulants.

## **Inhibitors and stimulators of germination of *Orobanche* (Mayer)**

Nurit Bar Nun, Tikva Dadon and **Alfred M. Mayer**

*Department of Botany*

*The Hebrew University of Jerusalem, Jerusalem 91904, Israel*

*E-mail: [mayer@vms.huji.ac.il](mailto:mayer@vms.huji.ac.il)*

**Introduction.** In seed biology, the existence of germination inhibitors and stimulators is well documented. Since *Orobanche* seeds germinate only after conditioning, their germination response shows some special features. Here we present some new data on the nature of some inhibitors and stimulators of the germination of broomrape.

**Results and Discussion.** Cells of the bacterium *Azospirillum brasilense*, a nitrogen fixing bacterium, contain an alcohol soluble, low molecular weight compound, MW of 1000 Da or less, which inhibits the germination and radicle growth of conditioned seeds of *Orobanche aegyptiaca*, when applied together with the germination stimulant GR24. Partial characterization of the compound suggests that it is a small peptide. At least two synthetic peptides, a linear and a cyclic one, had similar effects on the seeds as the factor extracted from and partially purified from cells of *A. brasilense*. We suggest that the factor present in *Azospirillum* and the synthetic peptides may act by competing for the site of binding of the germination stimulant.

Signaling molecules, which are involved in plant pathogen interactions, such as methyl jasmonate and methyl salicylate strongly inhibited germination even at very low concentrations,  $10^{-9}$  M

The stimulation of the germination of *Orobanche aegyptiaca* by smoke, derived from cellulose is described. The smoke dissolved in distilled water, was as effective as the germination stimulant GR24. High concentrations of the smoke were inhibitory. The active compound in smoke, described by others, is a butenolide having an unsaturated lactone structure. Coumarin, an unsaturated lactone, generally regarded as a germination inhibitor, is also able to stimulate germination, although radicle elongation was inhibited. When exposure was short and the concentration  $10^{-5}$  M, high germination and radicles appearing to be normal were obtained. The developing radicle of the *Orobanche* seed, treated with GR24 or smoke, contains starch grains along its entire length, which may be an indication of its ability to form an haustorium. Phenolics are also present along the radicle, but tend to concentrate at the radicle apex in germinating seeds, whose elongation is inhibited. This may also be an indication of haustorium formation

**Conclusions.** Compounds able to modify the germination of behaviour of *Orobanche* seeds include small peptides and signaling molecules which inhibit it, and smoke and coumarin, which can stimulate it. Inhibition or induction of premature germination of *Orobanche* could be used for the control of infection of hosts by the parasite. Hence these basic results have some practical implications.

**Points for discussion.** Comparisons of the similarities and differences between host infection by fungi and parasitic plants tend to stress the similarities. However, their appear to be fundamental differences, which should be considered in research for the control of parasitic plants

**Key reference.** A.M. Mayer. Pathogenesis by fungi and parasitic plants: similarities and differences. *Phytoparasitica* (In press).

## **Isolation and characterization of genes involved in the formation of the germination stimulants for the parasitic weed, *Striga* (Zhongkui Sun)**

**Zhongkui Sun**<sup>1</sup>, Reinhold Brettschneider<sup>3</sup>, Jules Beekwilder<sup>1</sup>, Eelco Hoogwout<sup>1</sup>, Ming Zhao<sup>1</sup>, Ton Bisseling<sup>2</sup> and Harro Bouwmeester<sup>1</sup>

<sup>1</sup>*Plant Research International, P.O. Box 16, 6700 AA Wageningen, The Netherlands*  
E-mail address of presenting author: [zhongkui.sun@wur.nl](mailto:zhongkui.sun@wur.nl)

<sup>2</sup>*Laboratory for Molecular Biology, WUR, Dreijenlaan 3, 6703 HA Wageningen (TB)*

<sup>3</sup>*University of Hamburg Biocentrum Klein-Flottbeck Ohnhorstrasse 18 D-22609 Hamburg GERMANY*

*Striga* and *Orobanchae* are the two major parasitic weed genera of the world causing large losses in many agricultural production systems. *Striga* spp. are found in Africa and Asia, and parasitise mainly maize, sorghum, cowpea and millet. *Orobanchae* spp. parasitise a large number of crops such as many legumes, crucifers, tomato, sunflower, hemp and tobacco and occur throughout the Mediterranean region, Eastern Europe and North Africa. Several authors have shown that the roots of the host species of *Striga* and *Orobanchae* excrete terpenoid-like germination stimulants that evoke germination of the seeds of the parasite. Although the germination stimulants so far identified were isolated from a wide variety of (host) crops, and induce germination of only distinctly-related parasitic weeds, the compounds themselves are strikingly similar. Nevertheless, nothing is known about the regulation of the biosynthesis of these compounds in the roots of the host species.

Our group recently elucidated that the germination stimulants of *Striga hermonthica* and *Orobanchae crenata* in maize, sorghum and cowpea are derived from the carotenoid pathway (Matusova *et al.*, 2005). Therefore, we intend to isolate and characterise key-genes in the formation of germination stimulants from this pathway. Here we use degenerate primers and RT-PCR to clone the target genes. These genes are characterised by *in vitro* expression system and transformation experiments. So far, a couple of genes have been cloned and characterised or are being characterised. The transgenic plants will be tested for an effect on the germination rate of *Striga* seeds.

### **Reference**

**Matusova R, Rani K, Verstappen FWA, Franssen MCR, Beale MH, Bouwmeester HJ** (2005) The strigolactone germination stimulants of the plant-parasitic *Striga* and *Orobanchae* spp are derived from the carotenoid pathway. *Plant Physiology*, in press

## **Influence of fluridone on conditioning and germination of *Orobanch* seeds (Joel)**

**DM Joel**<sup>1</sup>, D Plakhine<sup>1</sup>, SH Chae<sup>2</sup>, WJ Zhou<sup>3</sup>, K Yoneyama<sup>2</sup> and Y Takeuchi<sup>2</sup>

<sup>1</sup> *Newe-Ya'ar Research Center, ARO, Israel. [dmjoel@volcani.agri.gov.il](mailto:dmjoel@volcani.agri.gov.il)*

<sup>2</sup> *Center for Research on Wild Plants, Utsunomiya University, Japan*

<sup>3</sup> *College of Agriculture and Biotechnology, Zhejiang University, China.*

The germination of *Orobanch* seeds starts after chemical elicitation by germination stimulants that are released by host roots. Seed response to these stimulants is however possible only after conditioning, which is a developmental stage that starts after imbibition and lasts a few days. During this stage several metabolic processes take place in the seed, including respiration and GA synthesis.

Previous studies suggested that the presence of germination stimulants during conditioning inhibits the seed response to similar stimuli after conditioning. This inhibition limits our ability to use artificial stimulants as potential agents for false germination in the field.

Further research has shown that some known inhibitors of carotenoid biosynthesis, like Fluridone, prevent this stimulant inhibition, and also increase seed response to the germination stimulants, in various *Orobanch* species.

We further found out that fluridone stimulates the germination of *O. aegyptiaca* even without seed conditioning.

We therefore anticipate that the use of fluridone or similar compounds, alone or together with stimulants, may allow the stimulation of massive germination of *Orobanch* seeds in the field, and may thus be used for seed demise in soil.

## **Modelling seed dormancy and germination (Murdoch)**

Alistair J. Murdoch and Israel K. Dzomeku

*Department of Agriculture, The University of Reading, Earley Gate, Reading RG6 6AR, UK*

Email: [a.j.murdoch@reading.ac.uk](mailto:a.j.murdoch@reading.ac.uk)

Seeds of the parasitic weed, *Striga hermonthica*, have to undergo two processes before they will germinate in response to germination stimulants. These processes are afterripening and conditioning.

**Afterripening of air-dry (i.e. quiescent) seeds.** After seed maturation on the mother plant, the dry, viable seeds must undergo an afterripening process. Because of the low moisture contents of the seeds, this process occurs in *quiescent* seeds, i.e. seeds which are not actively metabolising. The process must therefore be primarily physical or chemical and occurs in the laboratory, when seeds are left on the bench at room temperature for several months. The process is analogous to the afterripening process of many other species in dry storage and can be modelled as a function of temperature. Moisture content of the seeds also affects the process although this aspect has not, to my knowledge been quantified or modelled. The rate of afterripening increases with increase in temperature with a  $Q_{10}$  of approx. 3. So increasing temperature from 20 to 40°C increases the rate of afterripening by a factor of approx. nine. Until seeds are afterripened they will not respond effectively to the next stage – (pre-)conditioning.

**Conditioning of afterripened, imbibed seeds.** Unlike afterripening, conditioning is a process that only occurs in seeds at high moisture contents and so seeds are actively metabolising. During conditioning, the sensitivity of the seeds to chemical stimulants increases to a maximum. The rate of the process increases with increase in temperature in the *Orobanche* and *Striga* species tested, but the temperature range over which the process can take place is limited by the ability of the seeds to survive at high temperatures. So the optimum is around 20°C in *Orobanche*. Imposing small amounts of water stress, changing temperature and including ammonium ions or urea during conditioning modifies the rate of conditioning in a way which can also be modelled. Results for the effects of nitrogenous compounds will be shown for *Striga hermonthica*. Prolonging the conditioning period often leads to an induction of secondary dormancy in imbibed seeds.

The non-linear empirical mathematical modelling approaches used by Kebreab & Murdoch (1999) to describe conditioning responses of *Orobanche* were tested on data for *Striga hermonthica*. Being a much more extensive data set than that available for *Orobanche*, the hypothesis that loss of primary dormancy is independent from induction of secondary dormancy could be tested for the first time and was rejected. Implications will be discussed. With calibration, the effect of delayed planting on emergence of parasitic weeds such as *S. hermonthica* could be modelled.

**Points for discussion.** No work has been carried out on afterripening of *Orobanche* seeds. Models for *Orobanche* are less well-developed than for *Striga hermonthica*. Are we aware of genes switched on/off during conditioning which make the seeds more sensitive to chemical stimulants such as strigolactones?

## **Processes and rates of *Striga hermonthica* seed bank depletion as a result of fallow and different crop covers (Van Mourik)**

T. A. Van Mourik

*Crop and Weed Ecology Group, Wageningen University, PO Box 430, 6700 AK Wageningen, The Netherlands*

*E-mail address: [tom.vanmourik@wur.nl](mailto:tom.vanmourik@wur.nl)*

Seed bank germination and depletion of the parasitic weed *Striga hermonthica* was measured at a site in Mali and a site in Niger during the 2004 rainy season under different crop and fallow systems. The seed burial and retrieval methods used were

- (1) mesh seed bags filled with sand and *Striga* seeds and
- (2) soil inoculation and sampling after which seeds were extracted by means of wet sieving and flotation.

Fates of exhumed seeds were assessed by a seed press test in which empty seeds were considered to have germinated.

Seed germination contributed most to seed bank depletion under a variety of vegetative cover types including host crops, non-host trap crops, intercrops of hosts and trap crops and weedy fallow. Low proportions (~10%) of exhumed seeds were infected by fungi. Most germination was found in the rhizosphere of the host crops sorghum (~70%, 140 DAS) and millet (~64%, 100 DAS), followed by the intercrops of a host and a non-host crop. Weedy fallow induced about the same germination proportions (~43-46%) as alleged trap-crops (~31-44%), indicating that induction of seed germination by other plants than cereals (hosts) may not have been species specific.

The soil sampling method and the seed bag burial method yielded similar percentages of seed bank depletion and treatment effects showed similar trends. Combining data from previous studies on seed production with these data on seed losses indicated that seed bank reduction by suicidal germination will only be achievable if seed production and seed bank replenishment are completely prevented.

**Point for discussion.** The results raise questions on the specificity of trap crops and whether differences reported previously in seed bank depletion between trap and host crops are simply caused by the prevention of seed production, rather than increased (suicidal) seed germination in the soil.

**Key words:** fallow, germination, host cereal, intercropping, parasitic weed, population dynamics, trap crop

## WGB: Resistance Breeding

### **Involvement of protein cross-linking, peroxidase and $\beta$ -1,3-endoglucanase in resistance of pea against *Orobanche crenata* (Pérez-de-Luque)**

**Alejandro Pérez-de-Luque**<sup>1\*</sup>, Clara I. González-Verdejo<sup>2</sup>, M. Dolores Lozano<sup>1</sup>, Miguel A. Dita<sup>1</sup>, José I. Cubero<sup>3</sup>, Pablo González-Melendi<sup>4</sup>, María C. Risueño<sup>4</sup> and Diego Rubiales<sup>1</sup>

<sup>1</sup> CSIC, Instituto de Agricultura Sostenible, 14080 Córdoba, Apdo. 4084, Spain

<sup>2</sup> IFAPA, CIFA Alameda del Obispo, 14080 Córdoba, Apdo. 3092, Spain

<sup>3</sup> ETSIAM-UCO, Dep. Genética, 14080 Córdoba, Apdo. 3048, Spain

<sup>4</sup> CSIC, Centro de Investigaciones Biológicas, Dep. Plant Development and Nuclear Organization, Ramiro de Maeztu 9, 28040 Madrid, Spain

\*Presenting author: [bb2pelua@uco.es](mailto:bb2pelua@uco.es)

**Introduction.** Root holoparasitic angiosperms, like *Orobanche* spp., completely lack in chlorophyll and totally depend on their host for their supply of nutrients. *O. crenata* is a severe constraint to legumes cultivation and breeding for resistance remains as the most economical, feasible and environmentally friendly method of control. Due to little resistance available in commercial pea cultivars, using wild relatives for breeding is necessary, and understanding the mechanisms underlying host resistance is needed in order to direct screening and breeding programmes.

Studies on the interaction between parasitic plants and their host are limited compared with other plant-pathogen systems. However, understanding the mechanisms underlying host resistance is needed in order to direct screening and breeding programmes. Several plant defence responses against microorganisms have also been identified against parasitic plants infection. At the molecular level, these responses include increased levels of phenolics and peroxidase activity (Goldwasser *et al.*, 1999; Pérez-de-Luque *et al.*, 2005a), induction of phytoalexins (Serghini *et al.*, 2001), lignification (Goldwasser *et al.*, 1999; Pérez-de-Luque *et al.*, 2005b), PR proteins (Joel and Portnoy, 1998), and 3-hydroxy-3-methylglutaryl CoA reductase encoding gene induction (Westwood *et al.*, 1998). Recent research using suppression subtractive hybridization (Vieira Dos Santos *et al.*, 2003) and proteomic techniques (Castillejo *et al.*, 2004) have led to identification of several genes expressed during the process of resistance to *Orobanche* spp. However, there is a long way to go in order to know which genes are really implicated in resistance to *Orobanche* and which of them are induced as part of a chain process: for example, chitinases could be induced by *Orobanche* infection, but these enzymes have effect only against fungi and not plant cell walls.

**Results.** In the present research, we have studied some of the factors involved in resistance to penetration of *O. crenata* in pea. Histochemical studies allowed us to determine that parasite intrusive cells were stopped within the host cortex, before reaching the central cylinder, and accumulation of H<sub>2</sub>O<sub>2</sub>, peroxidases and callose are detected in neighbouring cells. Protein cross-linking in the host cell walls appears as the mechanism of defence halting the parasite penetration. Based on these data and previous publications (Chang *et al.*, 1992; Joel and Portnoy, 1998; Goldwasser *et al.*, 1999; Vieira Dos Santos *et al.*, 2003; Castillejo *et al.*, 2004; Pérez-de-Luque *et al.*, 2005a) a peroxidase and glucanase/PR-2 genes, previously isolated

from *P. sativum*, were selected for *in situ* hybridization studies. They are expressed in cells of the resistant host near the penetration point during incompatible interactions and their role in the resistance to *O. crenata* is discussed.

## References

- Castillejo MA, Amiour N, Dumas-Gaudot E, Rubiales D, Jorrín JV.** 2004. A proteomic approach to studying plant response to crenate broomrape (*Orobancha crenata*) in pea (*Pisum sativum*). *Phytochemistry* **65**, 1817-1828.
- Chang M-M, Hadwiger LA, Horovitz D.** 1992. Molecular characterization of a pea beta-1,3-glucanase induced by *Fusarium solani* and chitosan challenge. *Plant Molecular Biology* **20**, 609-618.
- Goldwasser Y, Hershenhorn J, Plakhine D, Kleifeld Y, Rubin B.** 1999. Biochemical factors involved in vetch resistance to *Orobancha aegyptiaca*. *Physiological and Molecular Plant Pathology* **54**, 87-96.
- Joel DM, Portnoy, VH.** 1998. The angiospermous root parasite *Orobancha* L. (Orobanchaceae) induces expression of pathogenesis related (PR) gene in susceptible tobacco roots. *Annals of Botany* **81**, 779-781.
- Pérez-de-Luque A, Jorrín J, Cubero JI, Rubiales D.** 2005a. Resistance and avoidance against *Orobancha crenata* in pea (*Pisum* spp.) operate at different developmental stages of the parasite. *Weed Research* **45**, in press.
- Pérez-de-Luque A, Rubiales D, Cubero JI, Press MC, Scholes J, Yoneyama K, Takeuchi Y, Plakhine D, Joel DM.** 2005b. Interaction between *Orobancha crenata* and its host legumes: Unsuccessful haustorial penetration and necrosis of the developing parasite. *Annals of Botany* **95**, 935-942.
- Serghini K, Pérez-De-Luque A, Castejón-Muñoz M, García-Torres L, Jorrín JV.** 2001. Sunflower (*Helianthus annuus* L.) response to broomrape (*Orobancha cernua* Loeffl.) parasitism: induced synthesis and excretion of 7-hydroxylated simple coumarins. *Journal of Experimental Botany* **52**, 2227-2234.
- Vieira Dos Santos C, Delavault P, Letousey P, Thalouarn P.** 2003 Identification by suppression subtractive hybridization and expression analysis of *Arabidopsis thaliana* putative defence genes during *Orobancha ramosa* infection. *Physiological and Molecular Plant Pathology* **62**, 297-303.
- Westwood JH, Yu X, Foy CL, Cramer CL.** 1998. Expression of a defense-related 3-hydroxy-3-methylglutaryl CoA reductase gene in response to parasitization by *Orobancha* spp. *Molecular Plant-Microbe Interactions* **11**, 530-536.

## **Search for a scheme of host responses to *Orobanche* (Thoirin)**

**Séverine Thoirin**, Patricia Letousey, Philippe Delavault and Patrick Thalouarn  
*Faculte des Sciences et des Techniques, Univ. Nantes, 2 rue de la houssiniere,*  
*BP92208, F-44322 Nantes Cedex 3, France*  
[Severine.Thoirin@univ-nantes.fr](mailto:Severine.Thoirin@univ-nantes.fr)

Different host responses to *Orobanche* were studied in our lab, mainly using using gene expression analysis and histological approaches. Studies were performed on different susceptible plants (Arabidopsis, tomato and sunflower) and compared with resistant genotypes when available (LR1 sunflower resistant to *O. cumana*). Analyses performed on the three pathosystems (Arabidopsis or tomato/*O. ramosa*; sunflower/*O. cumana*), showed that hosts respond to *Orobanche* by inducing an array of general defence pathways such as phenylpropanoids, jasmonate and ethylene pathways, cell wall reinforcement, PR proteins and reactive oxygen species induction. Although some defences are induced after attachment of *Orobanche* to its host, defences are also induced very early, even if there is no physical contact between the host and the parasite, confirming emission of chemical signals by the *Orobanche* radicle and perception of these molecules by hosts (see Alexandre Lejeune's presentation). Moreover, the resistant sunflower line exhibited a stronger overall defense response against *O. cumana* than the susceptible genotype, involving marker genes of the salicylic acid pathway.

Perspectives concerning functional analysis of defense and perception of pathogens will be presented.

**Tomato/*O. ramosa* interaction:  
pathogen perception and defence elicitation (Lejeune)**

**Alexandre Lejeune**, Séverine Thoiron, Sabine Constant and Patrick Thalouarn.  
*Faculte des Sciences et des Techniques, Univ. Nantes, 2 rue de la houssiniere,  
BP92208, F-44322 Nantes Cedex 3, France*  
[Alexandre.Lejeune@univ-nantes.fr](mailto:Alexandre.Lejeune@univ-nantes.fr)

This work was undertaken to study defence reactions elicited by germinated broomrape seedlings on tomato.

A comparative study was made between tomato roots and cell suspension at the molecular and biochemical levels. These results gave evidence of secretion of an elicitor molecule by *Orobanche* even at an early stage of the interaction (i.e. prior to the attachment of *Orobanche* on to tomato roots).

Furthermore, the molecular approach revealed a tomato gene both induced in tomato cells suspension and roots. This gene which belongs to the wall associated receptor kinase family (*WAK*) could play a role in perceiving the parasite and thereby initiating a defense mechanism. To go further on this, we have conducted a wider-ranging study from the *WAK* gene to its product.

## **Sunflower genotypes resistant to the most virulent populations of broomrape in Romania (Păcureanu)**

**Maria Joița Păcureanu**, Matilda Ciucă and Steluța Raranciuc  
*Agricultural Research and Development Institute, Fundulea*  
*N. Titulescu st., no. 1, 915200, Fundulea, Romania*  
*e-mail: [mariapacureanu2@yahoo.com](mailto:mariapacureanu2@yahoo.com)*

**Introduction.** The parasite, *Orobanche cumana*, attacks sunflower on large areas from different countries in Eastern Europe and the Mediterranean basin as well as in Asia (Vrânceanu, 2000).

The first researchers who studied sunflower resistance to this parasite were Russian, who identified resistance sources as part of some sunflower genotypes. The monogenic, vertical resistance was established (Burlov and Kostiuk, 1976).

The different reactions of resistance in sunflower varieties of differing sensitivity to the pathogen in sunflower were reported during the last 20 years. (Ciriaev, 1987; Dominguez, 1996).

Resistance sources were identified in different wild, perennial sunflower species, but their introduction into cultivated varieties is difficult to do by classical methods.

In Romania, the parasite *Orobanche cumana* attacks sunflower on 45% from the cultivated area, six races having been identified (Păcureanu, 1998).

**Materials and methods.** The different sunflower genotypes (lines and populations) have been tested for resistance to broomrape attack, in order to identify new sources of resistance to the most virulent populations of this parasite and to establish a new differential set for parasite races from Spanish, Turkey and Romania.

The testing was performed under artificial inoculation using broomrape seeds obtained from two areas of infection in Romania, one in Spain and one in Turkey. Mitcherlich pots of 5 litres, with infested soil were used.

**Results and discussion.** As a result of breeding activity for resistance to broomrape attack, four pollen fertility restorer lines with complete resistance to this parasite were obtained by recurrent selection and two male cytoplasmic sterile lines were obtained by backcrossing.

The testing of resistance under artificial inoculation conditions, with different broomrape sources from Romania, Spain and Turkey has emphasized the different behaviour of some sunflower genotypes (lines and populations) to the attack of different broomrape sources.

- ❖ Odessa 507-1 line presented total resistance to broomrape population from Braila–Romania (F race) being sensitive to broomrape populations from Spain (F race).
- ❖ The Kr-3-2b line is totally resistant to the broomrape population from Spain (F race) and sensitive to the broomrape population from Romania-Braila (F race).
- ❖ The Mold-1-2 and Kiz 321-3-3b lines and VYP-70-4 and Alinka-1 populations were resistant to broomrape populations from Romania and Spain (F race) but sensitive to broomrape one from Turkey.

**Conclusions.** By different methods of breeding depending on type of resistance to broomrape attack, it is possible to obtain valuable sunflower lines, which could be utilized to obtain resistant hybrids. The different behaviour regarding the resistance to different broomrape populations has shown that the broomrape populations from Romania, Spain and Turkey are

different. The identified genotypes could contribute to the establishment of a new differential set for broomrape races.

**Opportunities for future collaboration.** The collaboration with the researchers from institutes in Cordoba-Spain, Edirne-Turkey, Novi-Sad-Serbia, Ukraine and Russia, will be continued in order to finalize a new differential set of broomrape races. As a partner, we will contribute to different European projects to solve the problems regarding the broomrape parasite.

#### **References.**

- Burlov., V.V., Kostiuk, S.V., 1976. Development of counterparts restoring pollen fertility and resistant to broomrape (*Orobanche cumana* Wallr.) and downy mildew (*Plasmopara helianthi* Novot.) Proc. 7<sup>th</sup> Intern Sunflower Conf., Krasnodar; U.S.S.R., Vol. I, 322-326.
- Ciriaev, P.V., 1987. Ustoicivosti samoopâlennîh linii podsolnocinika k zarazihe. Naucino-tehn. Biulleteni VNIIMK, Krasnodar, 11(97): 3-5.
- Dominguez, J., 1996. R-41, a sunflower restorer inbred line carrying two genes for resistance against a highly virulent Spanish population of *Orobanche cumana*. Plant Breeding, 115 (3): 203-204.
- Păcureanu-Joița, M., Vrânceanu, A.V., Soare, G., Marinescu, A., Sandu, I., 1998. The evaluation of the parasite-host interaction in the system *Helianthus annuus* L. - *Orobanche cumana* Wallr. in Romania. Proc. 2<sup>nd</sup> Balkan Symp. on Field Crops. Novi Sad, Yugoslavia, Vol. I: 153-155.
- Alexandru Viorel Vrânceanu, 2000. Floarea-soarelui hibridă. Edit. Ceres, București.

## **Resistance to new virulent *O. cumana* races (Fernández-Martínez)**

**José M. Fernández-Martínez**, L. Velasco and B. Pérez-Vich

*Instituto de Agricultura Sostenible, CSIC, Apartado 4084, E-14080 Córdoba, Spain*

*Email address of presenting author: [cs9femaj@uco.es](mailto:cs9femaj@uco.es)*

Broomrape (*Orobancha cumana* Wallr.), the most important parasitic angiosperm attacking sunflower (*Helianthus annuus* L.), is currently regarded as one of the most important constraints to sunflower production in Spain as well as in eastern European countries. This presentation deals with recent results, some of them not reported in previous meeting of the COST action, on the development of resistance to new virulent *O. cumana* races (F and G) and on the inheritance of resistance to race F.

Although the use herbicides is being considered as a promising control measure, at present, genetic resistance is the most effective and feasible control against *O. cumana*. However, the use of resistant cultivars has been frequently followed by the appearance of new pathogenic races overcoming the prevailing resistance genes, leading to an almost continuous need for new resistance sources. Until the early nineties, commercial hybrids cultivated in Spain were resistant to races A through E of broomrape. Resistance to races A through E was reported to be monogenic and dominant with five *Or* genes (*Or1-Or5*) providing an accumulative resistance to the five successive races A through E (Vranceanu *et al.*, 1980). From 1995, virulent populations of broomrape belonging to a new race, designated as F, have overcome all known resistance genes *Or1* to *Or5* in cultivated sunflower and have spread rapidly. More recently, a new race (designated race G), which overcomes race F resistant lines, has been identified (Molinero-Ruiz and Melero-Vara, 2005).

Resistance to broomrape populations belonging to the new virulent race has been found in wild and cultivated sunflower. Wild *Helianthus* species constitute the major source of resistance genes but cultivated germplasms are also valuable sources of resistance to race F of broomrape (Fernández-Martínez *et al.*, 2000). Accessions of more than twenty perennial species showed complete resistance and accessions of other species segregated for resistance to races E, F and G. Conversely, most of the wild annual species evaluated were susceptible. Four germplasm populations, BR1 to BR4, resistant to race F have been developed through interspecific hybridization of cultivated susceptible material and the perennial wild resistant species *H. divaricatus*, *H. maximiliani* and *H. grossesserratus*. These lines were registered in *Crop Science* and distributed to sunflower breeders (Jan *et al.*, 2002). The high level of resistance found in the evaluation of wild species contrasts with the results in cultivated material. Most of the accessions evaluated were susceptible to race F and only a low proportion showed full resistance or segregated. Several cycles of disease screening and selection resulted in the development of four lines, P-96, K-96, R-96 and L-86, uniformly resistant to races B, E and F and susceptible or showing segregation to race G and AM-1, AM-2 and AM-3 showing quantitative resistance to race F. All these lines have also been registered in *Crop Science* (Fernández-Martínez *et al.*, 2004; Pérez-Vich *et al.*, submitted). Resistance to Spanish broomrape populations belonging to race F has been also reported by several seed companies. Virulent races overcoming race E resistant lines, were identified in Rumania and Turkey and designated also as race F. However, preliminary unpublished studies indicate that the new races from Spain, Romania and Turkey have different degrees of virulence.

**Genetic studies** have shown that the resistance to race F in the material derived from cultivated sources was recessive and that two genes were involved. Conversely, the resistance

in BR4, one of the populations derived from wild species, is under the control of a single dominant gene. Moreover, the evaluation of crosses between race F resistant lines and different susceptible parental lines have shown that dominance relationships and genetic control of broomrape resistance in sunflower is highly dependent on the race of broomrape, the source of resistance, and also the susceptible parental line used for the cross (Pérez-Vich *et al.*, 2004a). Molecular studies aimed to map and characterize Quantitative Trait Loci (QTL) for resistance to races E and F were carried out (Pérez-Vich *et al.*, 2004b). Phenotypic variance for race E resistance was mainly explained by a major QTL associated to the resistance or susceptibility character, while race F resistance was explained by QTL with a small to moderate effect mainly associated with the number of broomrapes per plant, suggesting the existence of a quantitative component in the resistance to race F.

**In conclusion**, the rapid evolution of *O. cumana* populations leading to the appearance of new virulent races, requires a continuous search for new resistance sources. Results of evaluation of sunflower germplasm for resistance to different races have demonstrated that wild *Helianthus* species constitute the major source of resistance genes conferring resistance to new virulent races but cultivated germplasms are also valuable sources of resistance. Most of the resistant sources have been found to be controlled by major genes although quantitative resistance has also been reported. The combination of these two types of resistance could contribute to the development of a more durable resistance. The type of inheritance and dominance relationships are very important in breeding for broomrape resistance and hybrid production. Molecular marker studies should contribute to clarify the genetic control of broomrape resistance in sunflower and will constitute a powerful tool for breeding this trait.

## REFERENCES

- Fernández-Martínez, J.M., J. Melero-Vara, J. Muñoz-Ruz, J. Ruso and J. Domínguez, 2000: Selection of wild and cultivated sunflower for resistance to a new broomrape race that overcomes resistance to Or5 gene. *Crop Sci.* 40: 550-555.
- Fernández-Martínez, J.M., B. Pérez Vich, B. Akhtouch, L. Velasco, J. Muñoz-Ruz, J.M. Melero Vara, and J. Domínguez. 2004. Registration of four sunflower germplasms resistant to race F of broomrape. *Crop Sci.* 44:1033-1034.
- Jan, C.C., J.M. Fernández-Martínez, J. Ruso and J. Muñoz-Ruz. 2002. Registration of four sunflower germplasm populations resistant to broomrape race F. *Crop Sci.* 42:2217-2218.
- Molinero-Ruiz, M.L., and J.M. Melero-Vara. 2005. Virulence and aggressiveness of sunflower broomrape (*Orobanche cumana*) populations overcoming the Or5 gene. p. 165-169. In Seiler, G.J. (ed.), Proc 16<sup>th</sup> Int. Sunflower Conf., Fargo, ND, August 29-September 2, 2004. Int. Sunflower Assoc., Paris.
- Pérez-Vich, B., B. Akhtouch, A. Mateos, L. Velasco, C.C. Jan, J. Fernández, J. Domínguez, and J.M. Fernández-Martínez. 2004a. Dominance relationships for genes conferring resistance to sunflower broomrape (*Orobanche cumana* Wallr.). *HELIA* 27(40): 183-192
- Pérez-Vich, B., B. Akhtouch, S.J. Knapp, A.J. Leon, L. Velasco, J.M. Fernández-Martínez and S.T. Berry. 2004b. Quantitative trait loci for broomrape (*Orobanche cumana* Wallr.) resistance.. *Theoretical and Applied Genetics.* 109: 92-102.
- Pérez-Vich, B. L. Velasco, J. Muñoz-Ruz, J. Domínguez, and J.M. Fernández-Martínez. 2005. Registration of Three Sunflower Germplasms with Quantitative Resistance to Race F of Broomrape. *Crop. Sci.* (submitted)
- Vrânceanu, A.V., V.A. Tudor, F.M. Stoenescu, and N. Pirvu. 1980. Virulence groups of *Orobanche cumana* Wallr., differential hosts and resistance sources and genes in sunflower. In Proc. 9<sup>th</sup> Int. Sunflower Conf., Torremolinos, Spain, 8-13 July 1980. Int. Sunflower Assoc., Paris.

## Mapping of quantitative trait loci for resistance to *Orobanche crenata* in pea (Fondevilla)

Sara Fondevilla<sup>1\*</sup>, Diego Rubiales<sup>1</sup>, Zlatko Satovic<sup>2</sup>, María T. Moreno<sup>3</sup>, Ana M. Torres<sup>3</sup>

<sup>1</sup> CSIC, Instituto de Agricultura Sostenible, Apdo. 4084, E-14080 Córdoba, Spain

<sup>2</sup> Department of Seed Science and Technology, Faculty of Agriculture, Zagreb, Croatia

<sup>3</sup> IFAPA, CIFA-Alameda del Obispo, Apdo. 3092, E-14080 Córdoba, Spain

\*Presenting author: [cr2foaps@uco.es](mailto:cr2foaps@uco.es)

**Introduction** *Orobanche crenata* (crenate broomrape) is a root parasite that represents the major constraint to pea production in Mediterranean areas (Rubiales *et al.*, 1999). Only incomplete levels of resistance to broomrape have been identified in pea germplasm so far, suggesting that it is a polygenic trait. Higher levels of resistance have been found in non-adapted accessions of pea (Rubiales *et al.*, 1999; 2005; Pérez de Luque *et al.*, 2005). Thus, knowledge of the genomic location and linkage to molecular markers of these genes would facilitate gene transfer to pea cultivars through marker- assisted selection (MAS).

**Material and methods:** A population consisting of 111 RILs (Recombinant Inbred Lines) derived from a cross between an accession of *Pisum sativum* spp. *syriacum*, which is partially resistant to *O. crenata*, and the susceptible pea cv. Messire have been analysed and a linkage map has been developed. These RILs were evaluated against broomrape under field conditions in Córdoba (Spain). Resistance to *O. crenata* was scored as the final number of emerged *O. crenata* shoots per pea plant per RIL. Simple regression was carried out using the score of vigour of each RIL family as an independent variable and the *O. crenata* score as a dependent variable. The regression corrected values (residuals) were used to correct for the possible differences in vigour. Regression residuals, considered as the *O. crenata* index, were multiplied by -1 in order to assign greater values to the more resistant plants and a constant (10) was added in order to avoid negative values.

**Results:** The genetic map developed covers 1214 cM and contains three morphological, one isozyme, six STSs, six ESTs and 230 RAPD markers distributed in nine linkage groups. Of these, six groups have been assigned to chromosomes using markers common with the consensus pea map. A LOD of minimum 5.0 and a maximum recombination fraction of 0.3 (corresponding to a maximum Kosambi distance of 34.66 cM) were established as thresholds for grouping markers. Mean intermarker distance was 5.84 cM.

The parental lines showed substantial differences in broomrape resistance. The female parent (*P. sativum* spp. *syriacum*) displayed a resistant index of 11.6 corresponding to 0.42 *O. crenata* shoots per pea plant, while the male parent (cv. Messire) showed an index value of 5 corresponding to 11 *O. crenata* shoots per plant. The resistance indices of the RILs ranged between 3.5 and 13, with 10 being the average value. This index was successfully applied in the detection of one QTL involved in the resistance to this parasite. This QTL is located in chromosome IV. The peak value of LOD was 3.86. This putative QTL explained 19 % of phenotypic variation of this trait and showed additive genetic effect of 0.749.

**Future research** will be focused on studying the stability of this QTL in different environments and with different populations of the parasite. In addition, more accurate screening methods under controlled conditions might help to locate new QTL acting at different stages of the infestation process.

### References

- Rubiales D, Sillero JC y Cubero JI. 1999. Broomrape (*Orobanche crenata*) as a major constraint for pea cultivation in southern Spain. In: Cubero JI, Moreno MT, Rubiales D, Sillero J. (eds.) *Resistance to Orobanche: The state of the art*, pp. 83-89, Junta de Andalucía, Sevilla.
- Rubiales D, Moreno MT y Sillero JC. 2005. Search for resistance to crenate broomrape (*Orobanche crenata*) in pea germplasm. *Genetics Resources and Crop Evolution*. In press.
- Pérez de Luque A, Jorrín J, Cubero JI y Rubiales D. 2005. *Orobanche crenata* resistance and avoidance in pea (*Pisum* spp.) operates at different developmental stages of the parasite. *Weed Res.* 45, in press

## WG1: Taxonomy

### Genetic variation in *Orobanche foetida* as revealed

#### by AFLP analysis (Vaz Patto)

Maria Carlota Vaz Patto<sup>1</sup>, Ramón Diaz<sup>2</sup>, Belén Román<sup>2</sup> and Diego Rubiales<sup>3</sup>

<sup>1</sup> Instituto de Tecnologia Química e Biológica (ITQB), Plant Cell Biotechnology Lab, Apart.  
127, 2781-901 Oeiras, Portugal. email: [cpatto@itqb.unl.pt](mailto:cpatto@itqb.unl.pt)

<sup>2</sup> Departamento de Mejora y Agronomía, CIFA-Alameda del Obispo, Apdo 3092, 14080  
Córdoba, Spain.

<sup>3</sup> CSIC-Instituto de Agricultura Sostenible, Apdo. 4084, 14080 Córdoba, Spain.

**Introduction.** *Orobanche foetida* is commonly occurring in wild herbaceous leguminosae in the western Mediterranean area (Portugal, Spain, Morocco, Algeria and Tunisia) (Pujadas-Salvá, 1999). It has only been considered important as an agricultural parasite on faba beans (*Vicia faba*) and common vetch (*V. sativa*) in the Beja Region of Tunisia (Kharrat *et al.*, 1992). Nevertheless, very recently, *O. foetida* has been reported in Morocco (Saiss Region) also infecting common vetch (Rubiales *et al.*, 2005). This could represent a further constraint for legume production in this area.

The study of the genetic diversity among and between the *O. foetida* populations infecting the wild and cultivated plants of this region can elucidate the existing genetic relationships and suggest a potential origin for the recently detected vetch infecting population.

AFLP (amplified fragment length polymorphism) (Vos *et al.*, 1995) studies are widely used in genetic mapping studies but have proven also to be a competent tool in genetic diversity studies.

The aim of this work is to determine the genetic relationship among populations of *O. foetida* collected from wild and cultivated legumes in Morocco using AFLP markers.

**Materials and methods.** Three different populations of *O. foetida* collected on *Scorpiurus muricatus* and two infecting common vetch (*V. sativa*), in a total of about 50 individuals, were selected for AFLP analysis.

The AFLP technique was performed using the Analysis System I (Invitrogen) kit protocol with some modifications. The EcoRI primer was fluorescently labelled (Cys 5) to allow amplification products separation using an automatic sequencer ALF Express II (Amersham Biosciences).

Several different EcoRI/MseI based primer combinations (+3+3) and amplification conditions were tested. At the end, three AFLP primer combinations were selected based on the quality of amplification and used over the all *O. foetida* individuals.

Polymorphism scoring and analysis of genetic diversity is on the way.

#### References.

- Rubiales *et al.* (2005) *Plant Disease* 89(5): 528.  
Kharrat *et al.* (1992) *Fabis Newsletters* 30: 46.  
Pujadas-Salvá (1999) *Resistance to Orobanche: The state of the art*. Junta de Andalucía, Spain. pp: 187-193.  
Vos *et al.* (1995) *Nucleic Acid Research* 23(21): 4470-4414.

## **Preliminary results on genetic analysis of Greek *Orobanche* populations using RAPDs (Lyra)**

**S. Lyra**<sup>1</sup>, A. Katsiotis<sup>1</sup>, G. Economou<sup>2</sup> and P. Kaltsikes<sup>1</sup>

1. *Laboratory of Plant Breeding and Biometry*

2. *Laboratory of Agronomy*

*Agricultural University of Athens, Iera Odos 75, 11855 Athens Greece*

*e-mail: [sissylyra@yahoo.gr](mailto:sissylyra@yahoo.gr)*

**Abstract** In Greece, *Orobanche aegyptiaca* and *O. ramosa* are major constraints in tobacco and tomato fields in most areas. Broomrape plants were collected from naturally infected cultivated crops in two geographically isolated regions in the northern and western part of Greece. Four populations were sampled from each region and each population contained twenty individual plants, giving a total of 160 samples. *Orobanche ramosa* was mainly collected from tomato fields in western Greece, while *Orobanche* plants collected from northern Greece were present in tobacco fields. DNA was extracted following the standard CTAB protocol. A total of 60 RAPD primers were screened to test their efficiency and ten of them were selected based on their polymorphism. According to the results, both species *O. ramosa* and *O. aegyptiaca* are present in both areas and are well-separated in the RAPD derived phenograms. Furthermore, low genetic variability was observed within each population.

**Future work** There is a lot of work to be done on the discrimination of *Orobanche* species, since this research is the first systematic approach to study the inter/intra-specific variability of broomrape in Greece using molecular techniques. Our interest is basically focused on economically important species such as *O. ramosa*, *O. aegyptiaca*, *O. crenata* and *O. cumana* for the time being. In addition, the high phenotypic variability that is observed within each species needs further exploration, in order to examine if such variability is expressed on genetic basis.

### **Key references**

- Paran, I., Gidoni, D. and Jacobsohn, R. (1997). Variation between and within broomrape (*Orobanche*) species as revealed by RAPD analysis. *Heredity* 78: 68-74
- Román, B., Alfaro, C., Torres, A. M. , Moreno, M. T., Satovic, Z., Pujadas, A. and Rubiales D. (2003). Genetic Relationships among *Orobanche* Species as revealed by RAPD Analysis. *Ann. Bot.* 91: 637 - 642

## **Taxonomic changes in *Orobanche* and related genera (Rumsey)**

**Fred Rumsey**

*Department of Botany, The Natural History Museum,  
Cromwell Road, London, SW7 5BD*

[F.Rumsey@nhm.ac.uk](mailto:F.Rumsey@nhm.ac.uk)

This talk aims to summarise recent taxonomic changes within the Orobanchaceae from the familial to intraspecific level. Molecular studies have now clarified the long controversial relationship between the Orobanchaceae as traditionally delimited and the parasitic Scrophulariaceae. With the molecular support of a monophyletic parasitic lineage now regarded as a redefined and enlarged Orobanchaceae the morphological discontinuities identified by Boeshore (1920) and others are largely resolved. Molecular studies have also helped resolve questions relating to generic delimitation - with evidence now available to support cytological and morphological reasons for splitting the genus *Orobanche*. The progress towards a workable species level taxonomy and remaining problems will be considered.

## WG2: Biological control

### **Transforming *NEP1* toxin gene and other genes into two *Fusarium* spp. to enhance mycoherbicidal activity against *Orobanche* – failure, success and progress (Gressel)**

Ziva Amsellem, Sagit Meir, Olubukola Babalola, Hani Al-Ahmad, Einat Safran and  
**Jonathan Gressel**

*Plant Sciences, Weizmann Institute of Science, Rehovot, 76100, Israel*

[Jonathan.Gressel@weizmann.ac.il](mailto:Jonathan.Gressel@weizmann.ac.il)

**Introduction.** It is clear to us that pathogens attacking *Orobanche* are insufficiently virulent to provide the level of control demanded by growers. Superior formulations of viable inocula are prerequisites [1], but are not enough to overcome the evolutionary barriers preventing field wide control. Thus we have been transforming organisms we isolated [2] with various genes and “soft” genes such as those encoding auxin over-production increased virulence, but not enough [3]. We thus tested the *NEP1* gene encoding a fungal toxin from *Fusarium oxysporum* f. sp. *erythroxyli* that had successfully conferred hypervirulence when transformed into the *Colletotrichum coccodes* attacking *Abutilon theophrasti* [4].

**Materials and Methods.** *Fusarium oxysporum* (#CNCM I-1622) and *F. arthrosporioides* (#CNCM I-1621) were *NEP1*-transformed and assayed for hypervirulence on *Orobanche aegyptiaca* parasitizing tomato using methods described in [3, 4]. The co-transformation technology allows any number of genes to be simultaneously introduced.

**Results.** None of the *F. oxysporum* transformants were hypervirulent, but *F. arthrosporioides* killed *Orobanche* more quickly than the wild type. Transformed lines of both species were *NEP1* PCR positive, as was the wild type of *F. oxysporum* #CNCM I-1622 and five other forma speciales of *F. oxysporum*. All 6 wild type *F. oxysporum* excrete small amounts of immunologically detected *NEP1*-like protein. *NEP1* expression of most transformants was suppressed, suggesting that the native gene and the transgene silence each other. The sequence of the putative *NEP1* gene in *Fusarium oxysporum* #CNCM I-1622 differs from the sequence in the toxin over-producing strain in 5 (deduced) amino acids in the first exon. Wild type *F. arthrosporioides* does not contain the *NEP1* gene, explaining why it was amenable to transformation with high expression, and its virulence was enhanced due to a lack of a co-suppressing endogenous gene [5].

**Ongoing research.** As the ideal enhanced biocontrol agent will have a plethora of hypervirulence genes interacting with each other, hopefully in a synergistic manner, we have obtained a number of genes that we have evidence, or we presume will perform well. These include genes encoding ceratoplatinin, expansin, pectinase, and cellulase. The latter two are from bacterial sources and the genes are quite different from the fungal genes, to preclude the problems encountered with *NEP1*. The expansin gene is from a nematode. These genes are

presently being engineered into two versions of a universal expression cassette with multiple restriction sites that we have specially constructed for this purpose, one version with Trp C as the high expression promoter and the other Tox A promoter. Prior to transformations, we endeavoured to obtain preliminary evidence that the gene products would be active. For example, commercial pectinase and cellulase enhanced infection when sprayed with the fungi in the polybag system [6].

From a biosafety point of view, we are also planning on adding RNAi constructs against sporulation genes, to prevent the organisms from spreading beyond their points of application [7]. It is hoped that the addition of a large number of transgenes will cause the organisms to be quite unfit to compete for long periods against indigenous microorganisms, and will thus dissipate from the soil.

The constructs we are developing are available for collaborative research, subject to the standard constraints.

**Acknowledgements.** This research was supported as part of the EU 6<sup>th</sup> Framework Priority 5 - Food Quality and Safety Project: Enhancement and Exploitation of Soil Biocontrol Agents for Bio-Constraint Management in Crops (Contract no. FOOD-CT-2003-001687).

#### **Key References.**

- [1] Amsellem, Z., N. K. Zidack, P. C. Quimby, Jr. and J. Gressel (1999) Long term dry preservation of active mycelia of two mycoherbicide organisms. *Crop Protection* 18:643-649.
- [2] Amsellem, Z., Y. Kleifeld, Z. Kerenyi, L. Hornok, Y. Goldwasser, and J. Gressel (2001) Isolation identification and activity of mycoherbicide pathogens from juvenile broomrape plants *Biological Control* 21:274-284
- [3] Cohen, B.A., Amsellem, Z., Maor, R., Sharon, A., and Gressel, J. (2002) Transgenically-enhanced expression of indole-3-acetic acid confers hypervirulence to plant pathogens. *Phytopathology* 92:590-596
- [4] Amsellem, Z., B.A. Cohen, and J. Gressel (2002) Engineering hypervirulence in an inundative mycoherbicide fungus for efficient weed control. *Nature Biotechnology* 20:1035-1039
- [5] Amsellem, Z.S. Meir, H. Al-Ahmad, E. Safran and J. Gressel. Transforming *NEP1* into two *Fusarium* species to enhance mycoherbicide activity - failure and success. (submitted)
- [6] Babalola, O.O. and J. Gressel. Pectinase and cellulase enhance mycoherbicide activity of *Fusarium arthrosporioides* on *Orobancha aegyptiaca*. (in manuscript)
- [7] Gressel, J. (2001) Potential failsafe mechanisms against spread and introgression of transgenic hypervirulent biocontrol fungi. *Trends in Biotechnology* 19:149-154.

## **Compatibility of irrigation systems with the application of broomrape biocontrol agents (Vurro)**

**Maurizio Vurro & Angela Boari**

*Institute of Sciences of Food Production, National Council of Research, via Amendola 122/O,  
70125 Bari - Italy*  
[maurizio.vurro@ispa.cnr.it](mailto:maurizio.vurro@ispa.cnr.it)

Above or below ground drip irrigation is often used for vegetable crops, with several advantages for the plants and the environment, such as reducing water consumption and better management of nutrients. The development of the plant root systems is influenced by water, and roots tend to grow close to the water application systems. Microbiological control using irrigation systems might be an ideal way to control weeds and root pathogens, as microbes would be conveyed directly in proximity of the roots.

There might be great benefits from such methods of application, in terms of efficacy, reduced amounts of inoculum, protection from sources of inactivation (wind, UV light), no off-target spread and uniformity of distribution.

Considering that germination and underground growth are key phases for parasitic plant development, early application of microbial agents through the irrigation system could be very effective for the management of these very damaging weeds.

In order to verify the efficiency of supplying fungi by watering, a drip irrigation system has been developed in a greenhouse of about 30 m<sup>2</sup>.

Nine different dripper lines, among the most widely used in agriculture, were chosen for the tests. Each line is around 10 m long and contains between 25-50 drippers each. Each line was fixed along the bench, a few cm above the surface. To prevent drippers clogging, dish filters were used for each line. Each line was also connected to the main water line through a valve that allows switching the line on/off automatically or manually. Dish filters and valves were put together at the middle of the longest side of the greenhouse. To regulate the watering time, the volumes of treatments and the use of each line, a timer/controller was used. For injection of the conidial suspension into the lines a dosing pump has been used. This apparatus allows us to dilute and distribute the conidial suspension into the water flow as needed.

On the bench, plastic containers can be placed under the drippers to collect the spore suspensions. Aliquots of those suspensions were used for counting the number of spores collected and, using Petri dishes with suitable agarized media, also their viability.

Several experiments were carried out using several microorganisms, among which two species of *Fusarium* particularly promising for the biological control of *Orobanche ramosa*.

Different: watering times, amounts of water supplied through each irrigation line, amounts of spore suspension to apply through the system, were tested. The system set up and the results obtained will be presented.

## Use of fungal metabolites for broomrape suicidal germination (Vurro)

Angela Boari<sup>1</sup>, **Maurizio Vurro**<sup>1</sup>, Antonio Evidente<sup>2</sup>, Anna Andolfi<sup>2</sup>, Michele Fiore<sup>2</sup>  
<sup>1</sup>*Institute of Sciences of Food Production, National Council of Research, via Amendola  
122/O, 70125 Bari - Italy*

<sup>2</sup>*Department of Soil, Plant and Environment Sciences University of Naples Federico II, Via  
Università 100, 80055 Portici, Italy*  
[angela.boari@ispa.cnr.it](mailto:angela.boari@ispa.cnr.it)

Seeds of parasitic plants germinate only if stimulated by host root exudates and start producing a tubercle only if they are near enough to the host roots. After germination, the parasites have a long underground phase, and by the time they emerge, much of the damage has already been produced. Due to its unusual life cycle and the total dependence on the host, traditional control methods are very often impracticable.

On account of the dependence of the germination of seeds of parasitic plants on the presence of stimulating exudates produced by the roots of the host plant, an alternative approach for the management of parasitic weeds is the so called “suicidal germination”, that is, the induction of seed germination by the application of a germination stimulant to the soil, in absence of the host. The parasite seeds will germinate but, in absence of the host, will die, resulting in a reduction of the seed bank.

The chemical structure of some germination stimulants of species of another parasitic genus, *Striga*, is known but a few information are available for *Orobanche* species, with the exception of alectrol and orobanchol. Some natural compounds isolated from species both hosts and non hosts of *Striga* and *Orobanche* are known as strigolactones. Synthetic analogues of strigolactones named “GR” family have been developed and tested as well as several natural sesquiterpenes lactones. However the instability of strigolactones in the soil and their high costs for synthesis preclude up to now their practical use.

Among several fungal metabolites tested with the aim of finding new natural stimulants, fusicoccin and cotylenol proved to induce seed germination of *S. hermonthica* and *O. minor*.

Fusicoccin is the major toxic metabolite of *Fusicoccum amygdali*, the causative fungal agent of peach and almond canker. Considering

- (a) the efficacy of fusicoccin in stimulating seed germination of parasitic plants,
- (b) research in our laboratory to purify and identify several derivatives and natural analogues of fusicoccin and its aglycone and
- (c) the availability of those compounds in our lab,

a structure-activity study was carried out, using the seeds of *O. ramosa*. The results of this study will be shown.

## Natural enemies of *Orobanche* species in Slovakia (Tóth)

Peter Tóth, Kamil Hudec and Ľudovít Cagáň

Slovak Agricultural University, A. Hlinku 2, 949 76 Nitra, Slovak Republic

Email address of presenting author: [petery@nexta.sk](mailto:petery@nexta.sk)

**Introduction.** This contribution catalogues the organisms that have been recorded in association with the family Orobanchaceae in Slovakia during 2001 - 2005. It selects promising organisms and provides basic information on these potential biological control agents. In total, 22 insects and 17 pathogenic species associated with the Orobanchaceae have been recorded. Wasps, bees and flies are excluded where they are only casual and transient visitors to the plants. Although a relatively large number of species has been recorded from different *Orobanche* species, only a small number seem to have the potential for biological control of *Orobanche ramosa* and other pest broomrapes. As an addendum topics for future research on *Orobanche* concerning insects are described.

**Materials and methods.** The natural enemies of broomrapes (*Orobanche* spp.) were observed at crop fields and natural habitats in Slovakia during 2001 - 2005. There was only one broomrape, *Orobanche ramosa*, growing within the crop fields, while from among a variety of broomrapes growing in natural habitats three species were chosen as a model plants, *O. alba*, *O. flava* and *O. elatior*. Various locations within Slovakia were regularly checked and insects and pathogens recorded, collected, determined and laboratory tested. Pathogens were separated from the base and middle part of the stem fragments of infected *Orobanche* plants. The surface sterilized fragments were placed on Petri dishes with Potato-dextrose agar and incubated at 22 °C under 12/12 photoperiods (4000 lux, no UV light). The overgrown fungal colonies were isolated, purified and identified by visual and microscopic observation.

**Results.** A survey of insect on broomrapes (*Orobanche* spp.) carried out in Slovakia revealed 22 species belonging to the 10 different families. There were recorded, *Lygus rugulipennis*, *L. pratensis* (Heteroptera: Miridae), *Aphis fabae* Scopoli, *Myzus persicae* (Sulzer) (Sternorrhyncha: Aphididae), *Hypera postica* (Gyllenhal), *Otiorrhynchus scaber* (L.), *Anthonomus rubi* (Herbst), *Cionus tuberculosus* (Scopoli), *Dasytes niger* (L.) (Coleoptera: Dasytidae), *Nitidula* sp. (Coleoptera: Nitidulidae), *Isomira murina* (L.) (Coleoptera: Alleculidae), *Celypha lacunana* (D. & Sch.), *C. rivulana* (Scopoli), *Cnephasia stephensiana* (Doubleday), *C. genitalana* Pierce & Metcalfe, *C. asseclana* (D. & Sch.), *Argyrotaenia ljunghiana* (Thunberg) (Lepidoptera: Tortricidae), one species from family Geometridae (Lepidoptera), *Herminia grisealis* (D. & Sch.), *Diaphora mendica* (Clerck) (Lepidoptera: Noctuidae), *Phytomyza orobanchia* Kaltenbach (Diptera: Agromyzidae), *Chyliza extenuata* (Rossi) (Diptera: Psilidae). During the observations the most abundant were *P. orobanchia*, *M. persicae* and *Lygus* spp. feeding on each broomrape observed, *C. extenuata* noted from *O. alba*, *O. elatior* and *O. flava*, and *Celypha* spp. from *O. alba*, *O. elatior* and *O. flava*. The other species were less abundant. Only two flies, *P. orobanchia* and *C. extenuata*, and two moths, *D. mendica* and *Celypha* spp. caused significant damage.

Species composition of pathogens was observed basically on two wild broomrapes, *O. alba* and *O. flava*. The pathogens recorded were similar on both broomrapes, *Alternaria alternata*, *A. tenuissima*, *Aspergillus niger*, *A. flavus*, *Cladosporium cladosporioides* *Epicoccum nigrum*, *Fusarium oxysporum*, *F. sambucinum*, *F. solani*, *F. sporotrichioides*, *F. tabacinum*, *Rhizopus nigricans*, *Stemphylium botryosum*, *Penicillium* spp., *Phoma* spp., *Pythium* spp., *Trichoderma*

*spp.* The exceptions were *Aspergillus flavus*, *Cladosporium cladosporioides* and *Rhizopus nigricans* which were isolated only from *O. flava*.

The most important pathogen was *F. oxysporum*, where isolation frequency ranged from 25.8% - 72.7% in *O. alba* and from 31.8 - 43.7 in *O. flava*. In the case of sporulation (sporodochia formation) on the surface of flower heads under the natural conditions *F. oxysporum* (95%) had a dominant position followed by *F. sambucinum* (4%) and *F. solani* (1%).

**Conclusions.** The surveys that have been made give an indication of the kinds of insects and fungi which might be useful for biological control of broomrapes from Slovakia (Central Europe). Different investigations indicated the potential of native *P. orobanchia* populations to reduce *Orobanche* seed production under natural conditions, but the other insects recorded as natural enemies have not been studied sufficiently for their potential. Especially, *Chyliza extenuata* as a root feeder and *Diaphora mendica* as a seed capsule feeder seem to be very promising. Like *P. orobanchia*, *Fusarium oxysporum* f. sp. *orthoceras* is a well known natural antagonist of *Orobanche cumana*, but little is known about *Fusarium* spp. damaging *O. ramosa* as well about the efficiency of the whole spectrum of pathogens growing within broomrapes under natural conditions.

#### **Points for discussion or future research.**

- *P. orobanchia* is restricted in its host plant range, but morphological and behavioural differences reported in the literature suggest that populations in different regions may be adapted to particular host plant species. This should be explored. DNA analyses of *P. orobanchia* populations from different regions should be essential.
- Evaluate possible role of secondary metabolites emitted by broomrapes in attraction of *P. orobanchia* (insects).
- Utilize secondary metabolites emitted by broomrapes for mass trapping and redistribution of flies.

## **Biocontrol agents for *Orobanche* - A seaweed product: a new potential germination stimulant for *Orobanche ramosa* (Economou)**

S. Lyra<sup>1</sup>, G. Economou<sup>1</sup>, A. Anastasiadou<sup>1</sup>, A. Meletiou<sup>1</sup> and E. Kotoula-Syka<sup>2</sup>

1. Agricultural University of Athens, Laboratory of Agronomy  
Iera Odos 75, 11855 Athens

2. Democritus University of Thrace, Orestiada

E-mail address: [economou@aua.gr](mailto:economou@aua.gr)

**Introduction.** *Orobanche ramosa* constitutes one of the most serious parasitic weeds in tobacco and tomato crops in Greece, resulting in severe yield losses in most cases. Although different control methods for broomrape have been proposed from time to time (i.e. cultivation methods, solarization, fumigation, biological and chemical control, etc.), a considerable number of research activities have been directed towards the introduction of natural or synthetic chemical stimulants into the soil in order to induce germination in absence of the host. This control strategy is known as the “suicidal germination” approach. The *Orobanche* seeds germination by stimulants has been extensively stated as a promising method for the control of the holoparasite. GR24, a synthetic strigol-analogue, has been proved the most reliable agent to evaluate the seed germinability. Furthermore, the effect of growth regulators (i.e. cytokinins, gibberellins, auxins etc.) as germination stimulants has been well documented with satisfying results in some cases. From the physiological point of view, the above referred substances play an important role in certain plant functions such as seed germination, production of chlorophyll, resistance in environmental stress, etc. Based on these two approaches, the efficacy of a natural product, extracted from the alga *Ascophyllum nodosum* (*An* extraction), was evaluated for the induction of germination of *O. ramosa* seeds, suggesting its potential use as a germination stimulant for broomrape control.

**Materials and methods.** *O. ramosa* seeds were collected in central Greece from plant populations (A, B, C, D, E, F) parasitizing tobacco; populations A/B, C/D, and E/F were collected during the years 2002, 2003 and 2004 respectively. The seed germinability was evaluated using aqueous solution of *An* extraction at the following concentrations: 2.5 v/v, 1.25 v/v, 0.313 v/v, 0.078 v/v, 0.019 v/v, 0.0048 v/v, 0.0012 v/v. GR24 was used as a control at 3 ppm. *A. nodosum* has been a well-known alga for its enriched synthesis with optimum benefits for the plant growth at different phases in their life cycle. In particular, it is composed of growth regulators (IAA, cytokinins, gibberellins, etc.), aminoacids (proline, leucine, etc.), macro/micro-elements (N, P, K / Fe, Zn, B etc.) and organic matter (proteins, carbohydrates etc). The seeds were also treated with growth regulators contained in *An* product at the concentrations registered by the producer company; IAA (0.01 %), cytokinins (0.02 %), betaines (0.04 %), gibberellins (0.0025 %). The *Orobanche* seeds were firstly preconditioned at 23°C for 12 days before being subjected to the solutions mentioned above. The Petri dishes were then incubated at 25°C and observed periodically. Each treatment was conducted at three replicates.

**Results and Discussion.** The effectiveness of *An* extraction was evaluated on *Orobanche* seeds. The data showed that it may be characterized as stimulant triggering the germination of seeds at high levels in comparison to GR24 effect. Differences in germinability among the different concentrations of the solution indicated the response of the seeds to specific doses. In particular, some populations showed higher germinability values at lower concentrations (Table 1).

Table 1. Seed germination (G) and radicle growth (RL) of *O. ramosa* populations as influenced by *An* extraction at different concentrations and GR24 stimulant. Values within rows followed by the same letter are not significantly different at the 0.05 level as determined by the least significant means (LSM) by Tukey.

Populations	Germinability % Radicle length mm	2.5	1.25	0,313	0,078	0,019	0,0048	0,0012	H <sub>2</sub> O	GR24
		conc. v/v								
A <sub>2002</sub>	G	0,000	24,285cdef	38,874bcdef	39,814bcdef	78,510ab	32,646bcdef	31,723bcdef	14,969def	100,000a
	RL	0,000	2,098abc	2,824abc	1,940abc	2,330abc	2,662abc	3,693abc	3,147abc	2,591abc
B <sub>2002</sub>	G	0,000	17,43def	15,50def	13,54def	20,38def	14,92def	14,778ef	16,41def	31,540bcdef
	RL	0,000	3,39abc	3,78abc	4,67a	2,81abc	3,04abc	3,61abc	4,27ab	4,11ab
C <sub>2003</sub>	G	0,000	3,597ef	10,855def	3,307ef	65,85abc	36,467bcdef	13,487def	4,890ef	71,461abc
	RL	0,000	2,497abc	3,534abc	3,349abc	2,999abc	2,969abc	2,942abc	4,507ab	3,014abc
D <sub>2003</sub>	G	0,000	17,143def	19,880def	13,921def	15,090def	11,828def	9,095def	1,386ef	18,477def
	RL	0,000	2,334abc	3,137abc	3,354abc	2,755abc	3,215abc	3,053abc	2,079abc	2,786abc
E <sub>2004</sub>	G	0,000	2,778ef	5,595ef	5,757ef	60,75abc	8,484ef	2,795ef	0,913f	74,242ab
	RL	0,000	1,777abc	2,910abc	2,693abc	2,457abc	2,014abc	2,200abc	0,496c	1,517abc
F <sub>2004</sub>	G	0,000	18,119def	23,712cdef	28,105bcdef	20,562def	11,185def	16,848def	18,716def	33,731bcdef
	RL	0,000	1,989abc	1,932abc	1,942abc	1,461abc	1,979abc	1,374abc	1,151bc	1,112bc

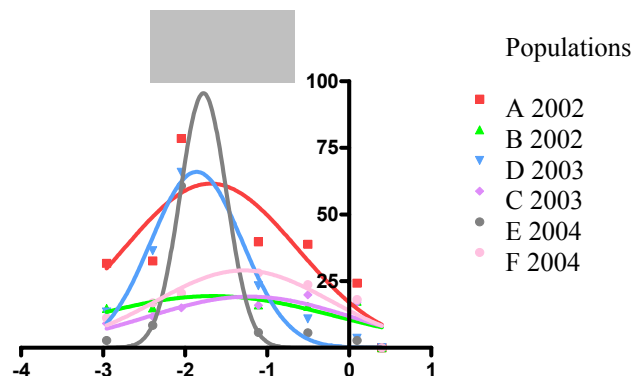
Table 2. Fit values (Area, SD and Mean) from germination curves, according to non-linear regression, of *O. ramosa* populations after *An* treatment

	2002		2003		2004	
	A	B	C	D	E	F
Area	162,7	73,6	59,74	91,8	67,62	77,41
SD	1,054	1,511	1,240	0,5548	0,2823	1,063
Mean	-1,687	-1,659	-1,217	-1,859	-1,776	-1,286

Measurements of radicle lengths showed high values and in some instances higher than those obtained from GR24 treatment. The assessment of the effectiveness based on seed germination curves, through the Non Linear Regression Analysis (NLR), indicated its increased influence on the populations A, D and E (Fig. 1). In contrast, seeds from B, C and F populations showed lower response to *An* application. However, the effect of *An* extraction varied significantly among the populations. It is particularly shown that the estimates derived from NLR analysis (area, SD) varied significantly in the case of the populations with high germinability, while there were no significant differences among the populations with low germinability (Table 2). The treatments with growth regulators (IAA, gibberellins, cytokinins) proved less effective compared to the *An* extraction, concluding that the latter may be considered as stimulating agent in this particular formulation.

**Conclusions and future outlook.** In general, the data obtained suggest that *An* product may have seed stimulant activity comparable with the synthetic strigol-analogue GR24. Although *in vitro* studies showed encouraging results for *O. ramosa* seeds germination, additional research is needed in order to study the efficacy of *An* extraction on other *Orobanchae* species (work in progress). It is also reasonable to assume that the specific mechanism of stimulation by *An* needs further investigation. Upcoming studies should also focus on *An* evaluation via field trials, in order to detect its possible efficacy to control broomrape. Moreover, its influence on the increase of the radicle length should be experimentally evaluated through the parallel use of trap or catch crops from the integrated point of view. In conclusion, it is apparent that our plans for future research are multi-faceted.

Fig. 1 Germination curves for six populations of *O. ramosa* seeds after the treatment of NNP at the conc. 2.5, 1.25, 0.313, 0.078, 0,019, 0,0048 and 0,0012 v/v. Lines were fitted according to Non Linear Regression Analysis.



### Key References

- Batchvarova, R. B., Slavov, S. B. and Bossolova, S.N. (1999). In vitro culture of *Orobanchae ramosa*. *Weed Research*, 39(3):191-197
- Bouwmeester, H. J., Matusova, R., Zhongkui, S. and Beale, M. H. (2003). Secondary metabolite signaling in host-parasitic plant interactions. *Current Opinion in Plant Biology*, 6:358-364
- Joel, D. M., Steffens, J. C. and Matthews E. (1995). Germination of Weedy Root Parasites in *Seed Development and Germination* eds. J. Kigel and G. Galili
- Kebreab, E. and Murdoch, A. J. (1999). A quantitative model for loss of primary dormancy and induction of secondary imbibed seeds of *Orobanchae* spp. *Journal of Experimental Botany*, 50:211-219.

## WG4: Integrated control and biodiversity conservation

### ***Orobanche crenata* control on legumes in various intercrops (Rubiales)**

Mónica Fenández-Aparicio<sup>1</sup>, Josefina C. Sillero<sup>2</sup> and Diego Rubiales<sup>1</sup>

1. Institute for Sustainable Agriculture, IAS-CSIC, Córdoba\*

2. IFAPA, CIFA Alameda del Obispo, Córdoba

\* IAS-CSIC, Apdo. 4084, E-14008 Córdoba, Spain. E-mail: ge2ruozd@uco.es

*O. crenata* is the most dangerous and the most widespread *Orobanche* species in the Mediterranean region and West Asia. It is a major constraint for faba beans, field peas, lentils, vetches and various forage legumes. Several control strategies have been employed but all without unequivocal success. The methods are either not feasible, uneconomic, hard to achieve or result in incomplete protection. The integration of several control measures is most desirable.

Intercropping maize or sorghum with cowpea and groundnut has been reported to reduce *Striga* infection, probably due to higher shading and increased fertility (1). Also intercropping maize with the perennial tropical legume *Desmodium* can reduce *Striga* infection by an inhibitory effect on haustoria formation (2). Such an inhibitory effect might be explored in our system. The objectives of the present studies were

- i) to explore the feasibility of control of broomrape by intercropping faba bean, lentils, grasspea and pea with fenugreek (*Trigonella foenum-graecum*), Egyptian clover (*Trifolium alexandrinum*) and cereals and
- ii) to discern the potential mechanisms of disease reduction.

*In vitro* studies showed that cereals, fenugreek and Egyptian clover induced very little broomrape seed germination compared to crop legumes which resulted in no infection. This indicates that they are unlikely to be effective trap or catch crops. However, they might be helpful when intercropped with host crops interacting with broomrape seed germination and/or haustoria formation, as well as on competing with host plants for soil and water. Intercropping lentil, grasspea or pea with fenugreek resulted in about 40% reduction in germination, and a reduction a bit higher (range 40-60% reduction) with Egyptian clover, which resulted in about 50% reduction of infection (number of broomrape tubercles formed per cm host root) in all host crops. This suggests that fenugreek and Egyptian clover roots exudates contain inhibitors of broomrape seed germination.

Pot experiments confirmed a reduced broomrape infection (two-threefold) on faba bean, pea and grasspea when intercropped with fenugreek and Egyptian clover at various proportions. Field experiments of pea and faba bean intercropped with fenugreek at various combinations confirmed the reduction in broomrape emergence/infection was about 50% in the combination 1:1.

Field experiments of faba bean and pea intercropped with oat, barley or triticale showed a significant reduction of infection in the intercrop. This reduction was confirmed in *in vitro* studies. Experiments to discern the mechanism of disease exclusion are being designed.

**References**

- (1) Tenebe V.A. and H.M. Kamara, 2002. Effect of *Striga hermonthica* on the growth characteristics of *Sorghum* intercropped with groundnut varieties. *J. Agronomy & Crop Sciences* 188: 376-381.
- (2) Khan Z.R., A. Hassanali, W. Overholt, T.M. Khamis, A.M. Hooper, J.A. Pickett, L.J. Wadhams and C.M. Woodcock, 2002. Control of witchweed *Striga hermonthica* by intercropping with *Desmodium* spp., and the mechanism defined as allelopathic. *Journal of Chemical Ecology* 28: 1871-1885.

## **Use of herbicide resistant crops in Greece for control of *Orobanche* and other weeds (Kotoula-Syka)**

<sup>1</sup>Eleni Kotoula-Syka and <sup>2</sup>Garifalia Economou  
<sup>1</sup>Democritus University of Thrace, <sup>2</sup>Agricultural University of Athens  
[kotoulaeleni@yahoo.gr](mailto:kotoulaeleni@yahoo.gr)

In Greece the main crops severely affected by broomrapes are tomato and tobacco and to a lower degree sunflower, beans, pea, chickpea and carrots. Most areas where these crops are grown have been infected by broomrape, to a varying and increasing degree, causing considerable yield losses.

Several control methods for the parasite have been described in previous studies. However all the methods are criticized as the parasite remains a great threat for many crops in the Mediterranean zone. In tomato and tobacco crops, several herbicides have been tested and the most effective of them were glyphosate, imazaquin and chlorsulfuron. However, the results of these experiments were not always consistent.

Among novel control methods, the breeding of resistant crops is partially utilised in Greece. In particular, 'resistant hybrids' of sunflower result in satisfactory control of the parasite. Sunflower is the third most affected crop by *Orobanche* and it is mainly grown in the northeast part of Greece. It is noticeable in this area that there is no application of herbicides to control broomrape; the parasite is effectively controlled using two resistant hybrids, Favorite (Ypsilon S.A.) and Odil (Pioneer).

In the legumes (bean, pea, chickpea and carrots) neither of the methods referred to above are applied (chemicals, resistant crops) to control broomrape, except by hand-pulling which is considered prohibitive from the economic point of view.

Since recent research has been directed towards the use of transgenic crops against parasitic weeds, genetically modified, glyphosate-resistant, processing tomato, was used in greenhouse experiments to investigate the possibility of effective control of this parasite. The results of these experiments indicated that the use of herbicide-resistant crops could be a solution to the *Orobanche* problem because they allow the use of higher rates of glyphosate, a non selective herbicide that has given excellent control of the parasite.

Experiments with transgenic tomato or other *Orobanche* host crops have not been documented in Greece for legal reasons. However, in the northwest part of Turkey, very close to the area of Greece where sunflower is grown, the *Orobanche* control in sunflower is obtained by the use of imidazolinone-resistant hybrids.

Another very serious problem in agriculture, apart from *Orobanche* parasitism, is the development of herbicide resistant weeds in areas where there is sequential and intensive use of the same herbicides or herbicides with the same mode of action. Herbicide resistant weeds have been reported in 22 European countries. Fifty eight species are involved, of which 37 are dicots and 21 monocots.

In large weed populations with sufficient genetic diversity, some rare weeds are naturally resistant to herbicide(s) because they have genetically endowed traits enabling them to survive, and then reproduce, at the herbicide rate used. Under continuous herbicide selection pressure, resistant individuals will quickly be selected and will become the most important component of the weed flora within a few years.

In Greece, so far, there are three grass species with resistant populations involving three modes of action, ACCase, ALS and PSII inhibitors, and three broadleaf weeds with resistant populations involving two modes of action ALS and PSII inhibitors.

The major problems are encountered in areas where monoculture and use of herbicides with the same mode of action were and are common practices. So, wheat is the most affected crop, where *Lolium rigidum*, *Avena sterilis*, *Phalaris brachystachys* resistant to ACCase inhibitors and/or to ALS and to PSII inhibitors, were found. Among dicots, *Papaver rhoeas*, resistant to ALS inhibitors, *Chenopodium album* and *Amaranthus retroflexus* resistant to PSII inhibitors were found in wheat and potato crops respectively. In most cases it is very difficult to rotate herbicides with different modes of action in the same crop in order to control the herbicide-resistant weeds, and sometimes it is also difficult to rotate crops or to use alternative methods to control these weeds.

Under such conditions herbicide resistant crops could be an effective means of controlling herbicide-resistant weeds, because they allow the use of non-selective herbicides or herbicides with different modes of action.

Although such herbicide-resistant crops could be a solution not only for the parasitic plants but also for the herbicide resistant weeds, however in Greece very little work has been done, and only for experimental reasons. The only trials that have been done, after those mentioned before for *Orobanche* control, were in 1998 by Monsanto with Roundup-ready cotton (glyphosate-resistant) and by Aventis with Star link corn (glufosinate-resistant). Both experiments gave very promising results but no more research on herbicide-resistant weeds has been done in Greece since then.

As far as we know, no genetically-modified, herbicide-resistant crop has been approved by the E.U. for use in any member country except glyphosate-resistant canola, which has not been authorized by the Greek Government.

#### References.

- Kotoula-Syka E., and I.G. Eleftherohorinos 1991. *Orobanche ramosa* L. (broomrape) control in tomato (*Lycopersicon esculentum*. Mill) with chlorsulfuron, glyphosate and imazaquin. *Weed Research* 31:19-27.
- Kotoula-Syka E., and A. Tsaftaris 1996. The use of transgenic glyphosate-resistant tomatoes permits efficient control of *Orobanche*. *Proc. of NATO Advanced Research Workshop (Regulation of Enzymatic Systems Detoxifying Xenobiotics in Plants)*. Chalkidiki, Greece.
- Kotoula-Syka E., A. Tal, and B. Rubin 2000. Diclofop-resistant *Lolium rigidum* from northern Greece with cross-resistance to ACCase inhibitors and multiple resistance to chlorsulfuron. *Pest Management Science* 56:1054-1058.
- Kotoula-Syka E., M. Sybony, I. Georgoulas, and B. Rubin 2000. Sulfonylurea herbicide resistance in corn poppy (*Papaver rhoeas*) from northern Greece. *Proc. of XI<sup>th</sup> International Conference on Weed Biology*. Dijon, France.
- Kotoula-Syka E. 2003. Evolution, distribution and mechanism of herbicide-resistant weeds in cereal crops in Greece. *Proc. of 7<sup>th</sup> EWRS Mediterranean Symposium*. Adana, Turkey.

## **New advances in chemical control of *Orobanche aegyptiaca* in tomato (Lande)**

**Tal Lande**, Joseph Hershenhorn, Gay Achdari and Hanan Eizenberg  
Department of Phytopathology and Weed Research, Newe Ya'ar Research Center, ARO,  
Ramat Yishay, Israel. Email: lande@volcani.agri.gov.il

**Introduction.** *Orobanche* control is complicated because:

- (a) it is effective only as a prophylactic treatment;
- (b) the parasite is directly connected to the host, therefore highly selective herbicides are needed;
- (c) if the systemic herbicide should reach the parasite through the conductive tissues of its host, the host must be selective to the herbicide without reducing its phytotoxicity; and
- (d) the parasite continuously germinates throughout the season.

Monitor (sulfosulfuron 750 g a.i. l<sup>-1</sup>) is labeled for *O. aegyptiaca* control in tomato in Israel. Studies that were conducted in pots under greenhouse conditions resulted in excellent *O. aegyptiaca* control in tomato with sulfonylurea herbicides (Eizenberg *et al.*, 2004).

**Material and Methods.** Studies were conducted under greenhouse and field conditions. All field studies were located in commercial tomato fields and included four replications. The herbicides Monitor and Cadre (imazapic 480 g a.i. l<sup>-1</sup>) were applied by a motorized back pack sprayer equipped with a T-jet 11015 nozzle delivering 200 l ha<sup>-1</sup> at pressure of 300 kPa. Monitor applications were followed by 300 m<sup>3</sup> technical irrigation by sprinklers.

**Conclusions.** Two (14 and 42 days after planting-DAP) or three (14, 28, 42 DAP) foliar applications of 50 g ha<sup>-1</sup> Monitor effectively controlled *O. aegyptiaca* in tomato. These treatments are effective only for 70-80 DAP. Application of the imidazolinone systemic herbicide Cadre on tomato foliage prevents fruit setting by damaging the reproductive system. Tomato flowers throughout the growing season, with fruit set peaking at 50 to 60 DAP. We have found that early treatments with Monitor followed by Cadre application after the fruit setting peak, completely controlled *O. aegyptiaca* in tomato without causing any damage to the yield.

**Point for discussion or future research.** Timing for herbicide applications should be modeled for increasing treatment efficacy. Developing such a model for optimizing timing for herbicide applications is now in progress.

**Key Reference.** Eizenberg, H., Goldwasser, Y., Golan, S., Plakhine, D., and Hershenhorn, J. (2004). Egyptian broomrape (*Orobanche aegyptiaca* Pers.) control in tomato with sulfonylurea herbicides - greenhouse studies. *Weed Tech.* 18:490-496.

## Lessons learned from integrated control of *Orobanche* in Cyprus (Vouzounis)

**Nicos Vouzounis**

*Agricultural Research Institute, Nicosia, Cyprus*

Email: [n.vouzoun@arinet.ari.gov.cy](mailto:n.vouzoun@arinet.ari.gov.cy)

Vegetable production is an important agricultural sector in Cyprus. Intensification and use of monocultures in agriculture have aggravated the spread of parasitic weeds and other soil-borne pests through contaminated seeds and by movement of contaminated tillage and harvest equipment.

*Cuscuta campestris* attacks alfalfa, melon and other vegetables. *C. monogyna* attacks grapes and occasionally citrus and olives. The estimated yield losses due to *C. campestris* averaged 10% during the 1990's in alfalfa, and total destruction of grapevines could be expected from heavy attacks by *C. monogyna*.

*Orobanche crenata* infests broad beans and peas, while *O. ramosa* attacks aubergine, cabbage, melon, potato and tomato among other crops. Heavy infestations lead to total crop failure in the mentioned crops. *O. ramosa* also caused about 50% yield losses in celery. Glyphosate was effective for *Orobanche* control in broad bean and celery (Americanos, 1983; 1991).

*Phytomyza orobanchia* is an abundant natural enemy of *Orobanche* in Cyprus but this alone does not lead to an effective solution of the problem and farmers favour the use of herbicides. However, reduced rates of glyphosate application or other herbicides are often phytotoxic to some crops (Mesa-Garcia *et al.*, 1984; Mesa-Garcia and Vasquez-Cobo, 1985; Vouzounis and Americanos, 1998). There is also the risk of developing resistance when low rates of the same herbicide are applied in successive years.

As in many other countries, the management of soil-borne pathogens and pests in Cyprus, especially in greenhouses, has traditionally been based on pre-plant soil fumigation with methyl bromide (Ioannou, 2000). Methyl bromide is the most common soil fumigant worldwide, due to its effectiveness on a wide spectrum of pests, including parasitic weeds. One application can kill virtually 100% of the seed bank, as demonstrated by local experience since 1992 and also by overseas experience (Parker & Riches, 1993). However, as the use of this chemical was banned recently, soil solarization has shown promising results as an alternative to methyl bromide in the Mediterranean region, where sunlight is abundant and the summer temperatures are high. In Cyprus, soil solarization has been under study since the 1980's, using tomato, eggplant, and watermelon as test crops. In open-field and low-tunnel production systems for tomato and eggplant, solarization treatments applied in 0.8 to 1m wide strips for seven to eight weeks during July-August, raised the maximum soil temperature by 10 to 12°C and reduced the population density of soil-borne diseases in soil by up to 95%. In addition, these treatments controlled most annual weeds, improved plant growth and increased fruit yield by 60-135%, compared to the untreated check (Ioannou, 1999). In greenhouse grown tomato, soil solarization for three and six weeks controlled *Orobanche* by 75% and 99%, respectively (Abu- Irmaileh, 1991; Ioannou 2000).

Work at the Agricultural Research Institute has led to the formulation of strategies, which successfully controlled *Orobanche* in several vegetables. In broad beans, two sprays of glyphosate, at 45 to 90 g a.i. ha<sup>-1</sup> controlled *Orobanche* effectively (Americanos, 1983). In cabbage, spraying twice with glyphosate at 60 to 100 g a.i. ha<sup>-1</sup> or imazaquin at 5 to 10 g a.i. ha<sup>-1</sup> controlled the parasite (Americanos and Vouzounis, 1995). In celery, two treatments of glyphosate at 40 to 50 g a.i. ha<sup>-1</sup> controlled *Orobanche ramosa* and allowed the

celery heads to attain full size (Americanos, 1991), while in tomato, eggplant, melon and watermelon *Orobanche* was completely controlled in the field by mulching the soil with black polyethylene sheeting on the day of transplanting (Vouzounis and Americanos, 1998; Vouzounis, 2002-2003). Olive pumice (by product of olive oil processing), incorporated into the field soil at 30 and 60 l per running meter of a 30 cm furrow prior to planting melon and watermelon seedlings, caused severe toxicity to both crops. Nevertheless, the reduction of *Orobanche* was similar to that observed in treatments covering the soil with black plastic (Vouzounis, 2002-2003).

Work summarized in this short review indicates that the use of herbicides for vegetable production can be readily replaced by soil solarization. In addition, covering the soil with black polyethylene just prior to planting vegetable seedlings gave the best results in reducing *Orobanche* infestation and increasing yield. In different crops, control of *Orobanche* requires an integrated strategy. Linke and Saxena (1991b) recommended a combination of solarization, application of herbicides and hand weeding with careful choice of cultivars and sowing times in order to manage *Orobanche* in legume crops. None of these methods gave complete control when used separately. For future research in Cyprus effort should be made to investigate the effectiveness of different sowing or planting dates of some crops e.g. melon, watermelon, potato and other vegetables for the control of *Orobanche*. In addition, soil amendments other than soil pumice should be tested against the parasite.

## References

- Abu-Irmaileh, B.E. 1991. Soil solarization controls broomrape (*Orobanche* spp.) in host vegetable crops in the Jordan Valley. *Weed Technology* 5: 575-581.
- Americanos, P. G. 1983. Control of *Orobanche* in Broad beans. *Technical Bulletin* 50, Agricultural Research Institute, Nicosia, 4p.
- Americanos, P.G. 1991. Control of *Orobanche* in celery. *Technical Bulletin* 137, Agricultural Research Institute, Nicosia, 5p.
- Americanos, P.G., and Vouzounis, N. A. 1995. Control of *Orobanche* in cabbage. *Technical Bulletin* 170, Agricultural Research Institute, Nicosia, 8p.
- Ioannou, N. 1999. Management of soil-borne pathogens of tomato with soil solarization. *Technical Bulletin* 205, Agricultural Research Institute, Lefkosia, 12 p.
- Ioannou, N. 2000. Alternatives to methyl bromide for the management of soil-borne pathogens in Cyprus. *Proc. International Workshop on Alternatives to Methyl Bromide for the Southern European Countries*, Ministry of Agriculture, Athens, pp. 42-45.
- Linke, K. H. and Saxena, M. C. 1991b. Towards an integrated control of *Orobanche* spp. in some legume crops. Progress in *Orobanche* Research, *Proc. Int. Workshop on Orobanche Research*, Obermarchtal, 1989 (eds. Wegmann, K. and Musselman, L. J.). Eberhard Karls-Universitat: Tubingen, pp.248-256.
- Mesa-Garcia, J., A. de Haro and L. Garcia-Torres. 1984. Phyto-toxicity and yield response of broad bean (*Vicia faba*) to glyphosate. *Weed Science* 32: 445-450.
- Mesa-Garcia, J. and A. Vasquez-Cobo. 1985. Problematica del uso del glifosato en habas (*Vicia faba* L.). para el control de jopo (*Orobanche crenata* Fork). *Informacion Technica Economica Agraria* 61: 52-56.
- Parker, C., and Riches, C. R. 1993. Parasitic Weeds of the World, Biology and Control. *CAB International*, Wallingford, U.K.
- Vouzounis, N. A., and Americanos, P. G. 1998. Control of *Orobanche* (Broomrape) in Tomato and Eggplant *Technical Bulletin* 196, Agricultural research Institute, Nicosia, 7p.
- Vouzounis, N. A. 2002-2003. Control of *Orobanche* in melon and watermelon. *Review* 2002-2003, Agricultural research Institute, Nicosia, pp.92-93.

## **Control of *Orobanche* on sunflower and tobacco crops in România (Jinga)**

**V. Jinga**, H. Iliescu, Alina Ionita, Viorica Stanescu  
*Research-Development Institute for Plant Protection, Bucharest;*  
*B-dul Ion Ionescu de la Brad, 8, OP. 18, 013813*  
e-mail: [vasilejinga@zappmobile.ro](mailto:vasilejinga@zappmobile.ro) ; [icpp@pcnet.ro](mailto:icpp@pcnet.ro)

Broomrape (*Orobanche spp.*) is one of the most dangerous parasitic plants for sunflower and tobacco crops in România.

In the sunflower fields the populations of weedy broomrapes consist of plants of *Orobanche cumana* Wallr, and in the tobacco, *O. ramosa* L. *O. cumana* Wallr was found and sporadically other species.

The methods of control and management for broomrape included: crop rotation, clean seeds, sowing dates, biological control, genetic resistance and herbicides.

During the last few years, on sunflower crops, *O. cumana*, has shown a marked spread, especially in the south eastern area of România. Under both artificial inoculation and natural contamination conditions, the behaviour of some sunflower cultivars was studied during parasitism by *O. cumana* as well as the influence of specific herbicides on the phanerogamous parasite.

In tobacco, this parasitic plant causes losses both in yield and quality. In order to diminish the negative effect of broomrape on tobacco yield, various herbicidal treatments were tested. In two experimental fields of tobacco, the efficacies of five herbicide treatments were tested (Treflan 48 EC + Glyforom RV, Stomp 330 EC + Glyfogan 480 SL, Treflan 48 EC + Basta EC, Stomp 330 EC + Assert 250 EC, Stomp 350 + Basta EC).

The best control of broomrape was with Treflan 48 EC (trifluralin 480 g l<sup>-1</sup>) preemergent 2 l ha<sup>-1</sup> + Glyforom RV (glyphosate 360 g l<sup>-1</sup>) post emergent 0.2 l ha<sup>-1</sup> + 0.3 l ha<sup>-1</sup> applied at 40 and 60 days from planting.

## **Minirhizotron- a new method for in-situ modeling of the underground development of *Orobanche* (Eizenberg)**

**Hanan Eizenberg**<sup>1</sup>, Joseph Hershenhorn<sup>1</sup>, Moshe Silberbush<sup>2</sup> and Jhonathan Ephrath<sup>2</sup>  
<sup>1</sup>*Department of Phytopathology and Weed Research, Newe Ya'ar Research Center, ARO,*

*Ramat Yishay, Israel. Email: [eizenber@volcani.agri.gov.il](mailto:eizenber@volcani.agri.gov.il)*

<sup>2</sup>*Wylar Department of Dryland Agriculture, Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Sede-Boqer, Israel*

**Introduction.** The root parasite *Orobanche* causes severe damage to vegetables and field crops. Most of the parasitism process takes place during the underground, not observable stages of the interaction between the parasite and the host plant.

**Material and Methods.** A minirhizotron camera was inserted into clear plexiglas minirhizotron observation tubes buried in broomrape infested soil enables to study *in-situ* aspects related to the host-parasite interaction. Such interactions include parasitism dynamics, chemical control and growth rate and influence on host root development. Developmental stages of germination, penetration into the host plant roots and the tubercles production were inspected repeatedly and recorded throughout the growing season by means of minirhizotron camera.

**Conclusions.** Using this nondestructive technique, the complete individual parasite life cycle (from germination to *Orobanche* shoot) was monitored. In addition, the response of *O. cumana* control in sunflower with the systemic herbicide Cadre (imazapic, 480 g a.i. l<sup>-1</sup>) was recorded and quantified.

**Point for discussion or future research.** This new technology should be adopted for using in several fields of studies such as resistance, chemical control, modeling parasitism dynamics, and interactions between the parasite and the rhizosphere.

***Rhinanthus minor* (Yellow rattle) –  
Grassland weed or the ecologist's friend? (Westbury)**

**Duncan B. Westbury**

*Centre for Agri-Environmental Research, Department of Agriculture, The University of  
Reading, Earley Gate, PO Box 237, Reading RG6 6AR, U.K.*

Email: [d.b.westbury@reading.ac.uk](mailto:d.b.westbury@reading.ac.uk)

It was not until 1847 that the French botanist Decaisne positively established that yellow rattle was a form of plant parasite capable of reducing harvests. Previously, Holland (1808) found that in Cheshire, yellow rattle was 'not in general liked by the farmer' and at the dawn of intensive industrial agriculture in Britain, the presence of yellow rattle was noted as doing 'a great deal of harm' in grassland 'owing to the fact that it kills or seriously weakens the grass plant' (Bastin 1915). Furthermore, Long (1924) noted that farmers sometimes complained of butter tasting sour when produced from cows grazing on pastures containing yellow rattle. With increasing evidence against this species as a permissible component of lowland grasslands, over the most part of the 20<sup>th</sup> century, "scientific" management of meadows included the eradication of this flower (Davies & Davies 1997). In turn this resulted in a dramatic decline in yellow rattle populations along with its semi-natural grassland communities.

Following the de-coupling of farming subsidies from production to practices encouraging farmland biodiversity, the presence of yellow rattle is now seen as a friend by both the ecologist and the farmer. Furthermore, the observation that the presence of yellow rattle can achieve significant reductions in the productivity of grasslands, and particularly of the dominant grasses (Davies *et al.* 1997), it now has a role in the processes of grassland diversification.

The abundance and persistence of yellow rattle is dependent on numerous factors of which, habitat management and operational timings are paramount. In this paper the promotion and control of yellow rattle is discussed in an effort to address whether parallels with *Orobanche* species can be drawn, leading to a greater understanding of *Orobanche* population dynamics.

### **References**

- Bastin, S L 1915 The warfare against weeds. *Journal of the Bath & West & Southern Counties Society*. 10: 59-70
- Davies A & Davies O 1997 English agriculturists' attitudes towards grassland vegetation, 1780-1914: an ecological perspective. *Land. Hist.* 18: 71-80
- Davies, D M, Graves, J D, Elias, C O & Williams, P J 1997 The impact of *Rhinanthus* spp. on sward productivity and composition: implications for the restoration of species-rich grasslands. *Biol. Conserv.* 82: 87-93
- Decaisne, M J 1847 Sur le parasitisme des *Rhinanthacées*. *Ann. Sci. Nat.*, Sér 3, 8: 5-9
- Holland, H 1808 *A General View of the Agriculture of Cheshire*. London
- Long, H C 1924 *Plants poisonous to livestock*. 2nd edn. Cambridge University Press, Cambridge

# Climate change

## **Predicting the impacts of climate change on crops (Wheeler)**

**Tim Wheeler**

*Crops and Climate Group, The University of Reading, Earley Gate, PO Box 237, Reading  
RG6 6AR, U.K.*

Email: [t.r.wheeler@rdg.ac.uk](mailto:t.r.wheeler@rdg.ac.uk)

Crops are inherently sensitive to weather and climate. Variability in climate can account for much of the variation in crop yields from year to year in many parts of the world. This is particularly so over the drier regions of the globe where the productivity of annual crops is heavily dependant on the rainfall received in the growing season. Extremes of weather also affect crops, whether these are hot temperatures experienced over Europe, a break in the monsoon rains over Africa or Asia, or the unexpected flooding of crop lands.

There is international scientific consensus, as expressed through the reports of the Intergovernmental Panel on Climate Change, that the world's climate is changing due to human activities. Emissions of greenhouse gases such as carbon dioxide and nitrous oxides are likely to lead to a warming of the global mean temperature by up to 6°C, and changes in rainfall patterns, by the end of the 21<sup>st</sup> century. It is a major challenge to the scientific community to try to predict the impacts of changes in climate on crop production, and to identify actions to mitigate any detrimental effects.

Much progress has been made in assessing the impacts of global climate change on crop production to date. This has included many crop experiments and simulation studies. Research is moving towards a consensus that predicts a small increase in production of the major grain crops across northern, developed nations up to 2050, followed by a gradual decline to 2100, and a steady decline in yields in tropical regions over this entire period. Thus, there will be a range of responses over the coming decades depending on geographic location. A uniform response is not expected across Europe either. Northern parts of Europe will see the challenges of adapting to crops or varieties that are favoured by the warmer conditions, and pests and diseases that perhaps are new to these areas. The availability of water for agriculture will most likely be the major challenge to farmers in Southern Europe.

Many research questions remain. These included: what are the direct effects of climate change on crops growing under field conditions; how can the output of climate models be best used in crop model simulations; and will different societies have the capacity to respond to the impacts of climate change on agriculture. The effects of variability in *current* climates on crops can be devastating. Crop production in some parts of the world is expected to become even more vulnerable to climate in the future.