

# IPPS



PROCEEDINGS

IPPS

International Parasitic Plant Society

10th WORLD CONGRESS OF PARASITIC PLANTS

8-12 June 2009, Kusadasi, Turkey

Organized by

Ministry of Agriculture and Rural Affairs

Ege University

Edited by

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MONSANTO



VESTA  
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## CONTENTS

INTERNATIONAL SCIENTIFIC AND ADVISORY COMMITTEE	2
LOCAL HOST COMMITTEE	2
LOCAL ORGANIZING COMMITTEE	2
FORWARD	4
SCIENTIFIC PROGRAM	5
CONTACT	11
INVITED PRESENTATIONS	12
EVOLUTION AND PHYLOGENY OF PARASITIC PLANTS	16
PARASITE BIOCHEMISTRY AND PHYSIOLOGY	25
ECOLOGY AND POPULATION BIOLOGY OF PARASITIC SPECIES	37
HOST-PARASITE COMMUNICATION	66
PARASITIC WEED MANAGEMENT	78
HOST AND NON-HOST RESPONSES TO PARASITISM	115
BREEDING FOR PARASITIC PLANT CONTROL	122
SPECIAL TOPICS 1: HEMIPARASITES	132
SPECIAL TOPICS 2: OROBANCHE CUMANA	141
AUTHOR INDEX	148

## FORWARD

The rate of progress in parasitic plant research has been accelerating in recent years. New approaches and resources have led to breakthroughs in our understanding of parasite evolution, biology, ecology, host-parasite communication, and host response to parasitism. The future holds even greater promise, but despite these advances, parasitic weeds continue to devastate crops in much of the world and farmers have few new tools with which to face this problem. Thus, parasitic plant researchers from every part of the world and representing a multitude of disciplines have gathered in Kusadasi, Turkey to discuss their latest findings and share ideas for how to further advance our understanding of these troublesome and fascinating organisms.

This volume contains the abstracts submitted to the 10<sup>th</sup> World Congress on Parasitic Plants, held from 8 to 12 June, 2009 at the Pine Bay Resort Hotel in Kusadasi. It represents a snapshot of world research on parasitic plants, the questions, the challenges, and the triumphs. We hope you enjoy these proceedings and the collegial interactions of the congress.

**Diego Rubiales**  
Program Chair

**Ahmet Uludag**  
Local Organizer

**Jim Westwood**  
President, International Parasitic Plant Society

# SCIENTIFIC PROGRAM

## 7 June 2009

16:00-18:00 Early registration

## 8 June 2009

08:00-10:30 Registration

10:30-11:00 Opening Ceremony

### 11:00-12:00 Opening lecture

Chair: D. Rubiales, J. Westwood, A. Uludag

**R. ZIMDAHL** The role of Ethics in Science

### 12:00-12:30 Invited presentation

**D. JOEL** Taxonomic and evolutionary justifications for considering  
*Phelipanche* as a separate genus

## LUNCH

### 14:00 – 14:40 Invited presentation

Chair: D. Joel

**M. A. SELOSSE** One way of forest plants to make their living in deep  
shade: eating mycorrhizal fungi...

### 14:40-16:00 Evolution and phylogeny of parasitic plants

Chair: D. Joel

- 14:40 – 15:00 **Westwood et al.** The Parasitic Plant Genome Project: A Massive Gene Discovery Project for the Orobanchaceae
- 15:00 – 15:20 **dePamphilis et al.** The Parasitic Plant Genome Project II: Large-scale EST sequencing of *Triphysaria*, *Striga*, and *Phelipanche*
- 15:20 – 15:40 **Bolin et al.** Molecular phylogenetic relationships and a revised taxonomy of the holoparasitic family Hydnoraceae
- 15:40 – 16:00 **Thorogood et al.** Host specificity and speciation in *Orobanche minor*

## COFFEE BREAK

### 16:20-18:30 Parasite biochemistry and physiology

Chair: J. Westwood

- 16:20 – 17:00 **PÉRON *et al.*** Molecular, biochemical and histological characterization of the sucrose-degrading enzymes involved in the sink-strength of *Phelipanche ramosa*
- 17:00 – 17:20 **Okazawa *et al.*** Metabolome analysis of *Orobancha minor* seed germination for selective control of parasitic weeds
- 17:20 – 17:40 **Aly *et al.*** Could plant viruses move from a host plant to the parasitic weed *Phelipanche*?
- 17:40 – 18:00 **Gui-Lin and Shang-Wu** Research progresses of *Cistanche deserticola* and *Cynomorium songaricum* in western China
- 18:00 – 18:20 **Almansoori *et al.*** Stable isotope ratios and mineral nutrient composition of *Cynomorium coccineum* and its halophytic host *Zygophyllum qatarense* in Bahrain

19:30 WELCOME COCKTAIL

## 9 June 2009

### 08:50-10:30 Ecology and population biology of parasitic species I

Chair: H. Bouwmeester

- 08:50 – 09:10 **Maass *et al.*** Pollination biology in the genus *Hydnora*
- 09:10 – 09:30 **Brasil *et al.*** Distribution and sex ratio of the holoparasite *Pilostyles ulei* Solms-Laubach (Apodanthaceae) in Serra do Cipo, Minas Gerais, Brazil
- 09:30 – 09:50 **Hristova *et al.*** Application of ISSR methods in studying broomrape (Orobanchaceae) biodiversity in South/South-Western Balkans
- 09:50 – 10:10 **Meulebrouck *et al.*** Putting things on their heads: host age thwarts establishment of the holoparasite *Cuscuta epithimum*
- 10:10 – 10:30 **Hoeniges *et al.*** Why are rare *Orobancha* species rare?

## COFFEE BREAK

## 10:50 – 12:30 Special topics 1: Hemiparasites

Chair: F. Kananpiu

- 10:50 – 11:10 **Dorka et al.** Rhythms of nutational movement and seasonal changes in jasmonate levels during the course of the year and under constant conditions in mistletoe (*Viscum album*)
- 11:10 – 11:30 **Girija et al.** Effect of host interaction on the phytochemical composition of *Helicanthus elastica*
- 11:30 – 11:50 **Seraj and Azary** Linking parasitic plant-induced host morphology to tritrophic interactions
- 11:50 – 12:10 **Domeignoz and Ceccantini** Modifications in wood anatomy caused by the mistletoe *Struthanthus vulgaris* in the host *Tipuana tipu* in Sao Paulo, Brazil
- 12:10 – 12:30 **Acic and Macukanovic-Jocic** Impact of hemiparasitic species *Rhinanthus minor* on grassland diversity

## LUNCH

## 14:00 – 16:00 Ecology and population biology of parasitic species II

Chair: P. Delavault

- 14:00 – 14:40 **RODENBURG et al.** Invasion, impact and possible integrated management of the facultative hemi-parasitic weed *Rhamphicarpa fistulosa* in rain-fed lowland rice
- 14:40 – 15:00 **Economou et al.** Assessing the role of abiotic factors on *Orobanche* infestation in Solaneous crops using GIS
- 15:00 – 15:20 **Bakar et al.** Population spread, host status and damage of crop plants and weed species by *Cuscuta australis* R.Br. in Johore, Malaysia
- 15:20 – 15:40 **Mehrvarz** Taxonomic revision of Orobanchaceae in Iran
- 15:40 – 16:00 **Qasem** Parasitic weeds, a possible threat to fruit and forest trees in Jordan

## COFFEE BREAK

## 16:20-18:20 Host-parasite communication

Chair: K. Yoneyama

- 16:20 – 17:00 **BOUWMEESTER** Strigolactones: signaling molecules with surprising activities
- 17:00 – 17:20 **Jamil et al.** Quantifying the relationship between strigolactones and *Striga hermonthica* under varying levels of nitrogen and phosphorus in rice (*Oryza sativa*)
- 17:20 – 17:40 **Plakhine et al.** Broomrape seed conditioning and response to germination stimulants in soil
- 17:40 – 18:00 **Yoneyama et al.** Qualitative and quantitative differences in strigolactone exudation between *Striga* tolerant and susceptible maize cultivars
- 18:00 – 18:20 **Kohlschmid et al.** Can formononetin induce germination of parasitic weeds?

18:20-19:20 **Poster session I**

## 10 June 2009

08.30-18.00 Field trip to Bozdag to see Lorentus on chestnuts and potato areas infested with orobanche (it is not time to see orobanche unfortunately) via old town Birgi. Lunch boxes will be provided at Golcuk, a nature beauty

## 11 June 2009

### 8:30 – 10:10 Parasitic weed management I

Chair: K. Al-Khatip

- 08:30 – 09:10 **EIZENBERG et al.** PICKIT- a decision support system for rational control of *Phelipanche aegyptiaca* in tomato
- 09:10 – 09:30 **Kanampiu et al.** Empowering smallholder farmers for integrated striga control in Africa
- 09:30 – 09:50 **Tittcomb et al.** How does *Desmodium uncinatum* control the parasitic plant *Striga*?
- 09:50 – 10:10 **Aksoy et al.** National broomrape project in Turkey

COFFEE BREAK

## 10:30-12:00 Parasitic weed management II

Chair: M. Vurro

- 10:30 – 10:50 **Elzein et al.** Innovations for scaling-up of *Striga* mycoherbicides application in Africa
- 10:50 – 11:10 **Ndambi et al.** Colonisation of *Striga hermonthica* and its host sorghum by the mycoherbicide *Fusarium oxysporum* f.sp. *strigae*
- 11:10 – 11:30 **Muller-Stoever et al.** Mycoherbicidal management of *Orobancha cumana*: observations from three years of field experiments
- 11:30 – 11:50 **Ghotbi et al.** Influence of nutritional regimes on *Fusarium oxysporum* (Isolates Iran-502 and-507) as a biocontrol agent of Egyptian broomrape (*Phelipanche aegyptiaca*)
- 11:50 – 12:10 **Uygun et al.** Natural *Phytomyza orobanchia* Infestations on broomrapes in Turkey
- 12:10 – 12:30 **Toth and Bouwmeester** Is *Phytomyza orobanchia* fastidious?

LUNCH

## 14:00-16:00 Host and non-host responses to parasitism

Chair: D. Rubiales

- 14:00 – 14:20 **Lis et al.** Global gene expression profiling during resistant and susceptible interactions of cowpea with *Striga gesnerioides*
- 14:20 – 14:40 **Hoeniges et al.** The secret of broomrape host-specificity
- 14:40 – 15:00 **Sugimoto and Ueda** Induction of phytoalexin biosynthesis in *Lotus japonicus* roots in response to *Striga hermonthica* attachment
- 15:00 – 15:20 **Yoshida and Shirasu** Multiple layers of nonhost incompatibility to *Striga hermonthica*
- 15:20 – 15:40 **Farah** The response of two legume crops (hyacinth bean and kidney bean) to the parasitism of field dodder (*Cuscuta campestris*)
- 15:40 – 16:00 **Lozano-Baena et al.** Resistance mechanisms to *Orobancha crenata* in the model legume *Medicago truncatula*: The isoflavonoid response

COFFEE BREAK

## 16:20-17:40 Special topics 2: *Orobancha cumana*

Chair: R. Batchvarova

- 16:20 – 16:40 **Thoiron et al.** Implication of HaDEF1 defensin in sunflower resistance to *Orobancha cumana*
- 16:40 – 17:00 **Evcil et al.** The mutation breeding for broomrape resistance in sunflower
- 17:00 – 17:20 **Pacureanu-Joita et al.** Resistance and sensitivity in the parasitic system *Helianthus annuus* - *Orobancha cumana*
- 17:20 – 17:40 **Pineda-Martos et al.** Genetic diversity of *Orobancha cumana* populations from Spain and Eastern Europe

## 17.40-18.30 Poster session II

20:00 CONGRESS DINNER

12 June 2009

### 08:30-10:10 Parasitic weed management III

Chair: J. Sauerborn

- 08:30 – 08:50 **Nemli et al.** Research on broomrape control in tomato fields in western Turkey
- 08:50 – 09:10 **Sinha and De** Management of parasitic weeds in Eastern India
- 09:10 – 09:30 **Haddad et al.** Integrated control of *Phelipanche ramosa* on potato in Syria
- 09:30 – 09:50 **Chinnusamy et al.** Integrated management of Chinese dodder (*Cuscuta chinensis*) in lucerne (*Medicago sativa*) and in *Amaranthus viridis* - a leafy vegetable
- 09:50 – 10:10 **Ahom et al.** Suppressing *Striga hermonthica* parasitism in *Zea mays* with *Sesamum indicum* and *Glycine max* and nitrogen fertilization in Benue State, Nigeria

COFFEE BREAK

### 10:30-11:50 Breeding for parasitic plant control

Chair: M. Kharrat

- 10:30 – 10:50 **Maalouf et al.** Stability of *Orobanche* resistance of faba bean lines in various environments
- 10:50 – 11:10 **Adeosun et al.** Evaluation of early and extra-early maize cultivars for their reaction to *Striga hermonthica* in the North-Western Nigeria
- 11:10 – 11:30 **Slavov and Batchvarova** Chemical mutagenesis and haploidy - combined approach for breeding broomrape resistant tobacco
- 11:30 – 11:50 **Goldwasser et al.** Screening of chickpea (*Cicer arietinum*) genotypes for Field Dodder (*Cuscuta campestris*) resistance

### 11.50-12.30 Closing

## **CONTACT**

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## **INVITED PRESENTATIONS**

## **1. The role of ethics in science**

R. L. ZIMDAHL

Professor Emeritus, Colorado State University, USA

Those engaged in agriculture possess a definite but unexamined moral confidence or certainty about the correctness of what they do. This paper examines the origins of that confidence and questions its continued validity. The basis of the moral confidence is not obvious to those who have it or to the public. In fact the moral confidence that pervades agriculture is potentially harmful because it is unexamined. This paper advocates analysis of what it is about agriculture and society that inhibits or limits agriculture. All engaged in agriculture should strive to nourish and strengthen the aspects of agriculture that are beneficial and change those that are not. To do this we must be confident to study ourselves, our institutions, and be dedicated to the task of modifying the goals of both.

## **2. One way of forest plants to make their living in deep shade: eating mycorrhizal fungi...**

M. A. SELOSSE

Centre d'Ecologie Fonctionnelle et Evolutive (CNRS, UMR 5175), Equipe Interactions Biotiques,  
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Most plant ecophysiology is shaped by symbioses in which the roots of ~90% of terrestrial plant species associate with soil fungi to form mycorrhizae. Fungi exploit soil mineral nutrients and water that are shared with the plant, and, as a reward, they receive carbon from the plant. Reversing this flow, several forest-understorey achlorophyllous plants, once thought to be saprophytic, are now shown to receive carbon from their mycorrhizal fungi. They are therefore best called mycoheterotrophs (MH). In the many investigated temperate MH species, among which many orchids, the associated fungi in turn form mycorrhizae with surrounding trees that are the ultimate carbon source of the entire system. Direct and indirect evidences support the latter point, especially isotopic methods. The ability of mycorrhizal to link different plants therefore allows inter-plant carbon transfers. Investigations on green forest-understorey plants, phylogenetically close to MH species, has revealed a similar, more cryptic, nutritional strategy: (i) they also associate to fungi, that are not usual partners of the taxon they belong to, but are also mycorrhizal on surrounding trees, and (ii) they receive carbon from their fungi, as shown by their stable isotope content, that adds up to their photosynthates. They thus gain their carbon via a combination of MH and photosynthesis (mixotrophy), a strategy reported for some orchids and some ericaceous plants (*Pyrola* spp.). Phylogenetic analyses suggest that, in orchid evolution, mixotrophy evolved in shaded habitats and preceded MH nutrition. Indeed, rare achlorophyllous mutants can be found in mixotrophic orchid species: they may represent intermediate states in evolution toward MH strategies. Our recent physiological investigations suggest why, however, transition to full heterotrophy is rarely successful in these mixotrophic lineages.

In Memory of Prof. Edward S. Teryokhin

### 3. Taxonomic and evolutionary justifications for considering *Phelipanche* as a separate genus

D. JOEL

Newe-Ya'ar Research Center, Agricultural Research Organization, Israel

All weedy broomrapes have traditionally been grouped within the genus *Orobanchae*, but divided into two sub-generic sections, Sec. *Trionychon* and Sec. *Orobanchae* (beck-Mannagetta 1930). Nevertheless, both morphological and karyological differences between the two sections have also been claimed to justify splitting the genus into two distinct genera. Teryokhin (1997) discussed this issue in his book on weedy broomrape, adding his own observations and concluding that splitting is a necessity. He therefore grouped the species of Sec. *Trionychon* under the separate genus *Phelipanche* Pomel. However, most researchers still kept the traditional terminology until recently, because there has been no evidence for the phylogenetic relations between these two sections.

However, recent phylogenetic studies by Schneeweiss et al. (2004, 2005) and Manen et al. (2004), based on DNA-sequences from the nuclear ribosomal DNA and from the plastid genome, revealed that *Orobanchae* is not monophyletic, but falls into two phylogenetically distinct clades that coincide with the two *Orobanchae* sections. Obviously these molecular results complement the formerly reported morphological findings, leading to the recent decision to finally split the genus *Orobanchae* into two distinct genera: *Orobanchae* and *Phelipanche*. As a result, we should now use the names *Phelipanche ramosa* (L.) Pomel (syn. *O. ramosa* L.), and *P. aegyptiaca* (Pers.) Pomel. (syn. *O. aegyptiaca* Pers.).

	<i>Orobanchae</i>	<i>Phelipanche</i>
Bracteoles	No bracteoles	<b>2</b> bracteoles under the calyx
Calyx	Divided into <b>2</b> lateral segments	Cylindrical or campanulate with <b>4-5</b> Teeth
Stems	Not branched	Branched or simple
Fruit	Style persists	Style falls off
	Lateral opening	Top opening
Chromosomes	X = <b>19</b>	X = <b>12</b>

- Beck von Mannagetta G. 1930. Orobanchaceae. In: Engler, A. (ed.) Das Pflanzenreich 96 (IV.261):1-348.
- Manen, J.F., Habashi, C., Jeanmonod, D., Park, J.-M., Schneeweiss, G.M. 2004. Phylogeny and intraspecific variability of holoparasitic *Orobanchae* (Orobanchaceae) inferred from plastid *rbcl* sequences. Mol. Phylogenet. Evol. 33: 482–500.
- Schneeweiss, G.M., Colwell, A., Park, J.-M., Jang, C.-G., Stuessy, T.F., 2004. Phylogeny of holoparasitic *Orobanchae* (Orobanchaceae) inferred from nuclear ITS sequences. Mol. Phylogenet. Evol. 30: 465–478.
- Schneeweiss G. M., Park J.-M., Manen J.-F., Colwell A. E. & Weiss-Schneeweiss H. (2005) Phylogenetic relationships of *Orobanchae* and related genera: evidence from molecular and karyological data. In: Burckhardt & Mühlethaler (eds): 8th GfBS Annual Conference, Abstracts 88. <http://www.senckenberg.de/odes/ODE-05-13.pdf>
- Teryokhin, E.S. 1997. Weed Broomrapes: Systematics Ontogenesis Biology Evolution. Aufstieg-Verlag, Germany

**EVOLUTION AND PHYLOGENY  
OF PARASITIC PLANTS**

#### 4. The Parasitic Plant Genome Project: A Massive Gene Discovery Project for the Orobanchaceae

J. WESTWOOD<sup>1</sup>, C. DEPAMPHILIS<sup>2</sup>, M. TIMKO<sup>3</sup>, J. YODER<sup>4</sup>, M. DAS<sup>1</sup>, B. GOWDA<sup>3</sup>,  
P. GUNATHILAKE<sup>4</sup>, L. HONAAS<sup>2</sup>, K. HUANG<sup>3</sup>, K. LIS<sup>3</sup>, L. SHEAFFER<sup>2</sup>, V.  
STROMBERG<sup>1</sup>, K. WALL<sup>2</sup>, N. WICKETT<sup>2</sup>, B. WU<sup>4</sup>

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<sup>2</sup>Penn State University, USA

<sup>3</sup>University of Virginia, USA

<sup>4</sup>University of California, Davis, USA

The Parasitic Plant Genome Project (PPGP) has the objective of sequencing expressed genes from three members of the Orobanchaceae: *Triphysaria versicolor*, *Striga hermonthica*, and *Phelipanche aegyptiaca* (syn. *Orobanche aegyptiaca*). These species represent the diversity of parasite strategies from facultative hemiparasite to obligate holoparasite and, together with the forthcoming sequence of the non-parasitic relative *Mimulus guttatus*, create a powerful framework for making inferences on the molecular evolutionary changes associated with parasitism. The project is sequencing cDNAs representing transcripts from key life stages of each parasitic species, starting from pre-haustorial root/radicle growth through flowering, with emphasis on stages associated with haustorial connections. Initially, specific tissue stages are being sequenced by high throughput 454 sequencing and will be supplemented by Sanger-based sequencing. Since the quantities of biological material for the microscopic early and post-vascular attachment stages are limiting, a linear amplification technique is being used to generate enough cDNA for sequencing. The data from this project will be available on a project website as well as general repositories such as Genbank. The availability of these sequences will facilitate research on parasite evolution, growth and development, population biology, and the creation of resistant hosts. The presentation will include discussion of the sequences from above-ground vegetative and floral tissues and an update on sequencing progress for other tissues.

## 5. The Parasitic Plant Genome Project II: Large-scale EST sequencing of *Triphysaria*, *Striga*, and *Phelipanche*

C. dePAMPILIS<sup>1</sup>, N. WICKETT<sup>1</sup>, J. WESTWOOD<sup>2</sup>, M. TIMKO<sup>3</sup>, J. YODER<sup>4</sup>, M. DAS<sup>2</sup>, B. GOWDA<sup>3</sup>, P. GUNATHILAKE<sup>4</sup>, L. HONAAS<sup>1</sup>, K. HUANG<sup>3</sup>, K. LIS<sup>3</sup>, L. SHEAFFER<sup>1</sup>, V. STROMBERG<sup>2</sup>, K. WALL<sup>1</sup>, B. WU<sup>4</sup>

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<sup>4</sup>University of California, Davis, USA

The Orobanchaceae comprises 84 genera and >2000 species that span the continuum of parasitism from facultative hemiparasites to fully non-photosynthetic holoparasites, and several species that have severe impacts on agriculture across large geographic regions. The goal of the Parasitic Plant Genome project is to compare transcriptomes across both the diversity of these parasites represented by *Triphysaria versicolor* (facultative hemiparasite), *Striga hermonthica* (obligate hemiparasite), and *Phelipanche aegyptiaca* (obligate holoparasite), as well as between and among stage-specific sequenced cDNAs. A closely related non-parasitic plant, *Mimulus guttatus*, whose genome is being sequenced for an independent project, completes a comparative framework to identify genes associated with the origin of parasitism, as well as genetic changes associated with the transitions between parasites with varying degrees of host reliance. Initial analyses have been performed to study the rate of synonymous substitutions (Ks) of paralogous genes in *Triphysaria* from a set of Sanger-sequenced ESTs. The distribution of Ks values of gene-pairs suggests that a large-scale gene duplication event, potentially a whole-genome duplication, occurred relatively recently in the history of *Triphysaria*, and may have given rise to many genes involved in parasite function. An analysis of available EST data from *Mimulus* reveals that the non-parasite does not share this duplication event, suggesting that the genome duplication is specific to parasites. Corroboration of this event can be investigated by the identification of genes retained following the duplication, and gene family phylogenetic analyses. The impact of high-throughput cDNA sequence data on the placement of the duplication relative to other Orobanchaceae will be addressed, as well as a summary of an initial screen for parasite-specific genes.

## 6. Molecular phylogenetic relationships and a revised taxonomy of the holoparasitic family Hydnoraceae

J. F. BOLIN<sup>1</sup>, T. J. MOTLEY<sup>1</sup>, E. MAASS<sup>2</sup>, L. J. MUSSELMAN<sup>1</sup>

<sup>1</sup>Old Dominion University, Department of Biological Sciences, Norfolk VA USA

<sup>2</sup>University of Namibia, Department of Biology, Windhoek Namibia

Using nuclear (ITS) and plastid (rpoB) DNA sequences, we present the first phylogeny of the holoparasitic family Hydnoraceae. The family contains two genera, *Hydnora* and *Prosopanche*, that were resolved as sister clades. The species are parasites on two unrelated plant lineages. The ancestral host association seems to have been in the members of the Fabaceae followed by a single host-shift to the genus *Euphorbia* spp. (Euphorbiaceae) in *Hydnora*. Phylogenetic assessment of morphological evolution of *Hydnora* revealed that rhizome shape is a homoplastic character, thus a poor character for defining intrageneric sections. In contrast, synapomorphies included osmophore position and tepal color. Within *Hydnora africana* s.l., cryptic species were discovered including a new species from southern Namibia and the recognition of *Hydnora longicollis* Welw. in Angola and northern Namibia at the species level. We have shown that the cryptic species can be distinguished by floral odor and floral metrics. Within the *Euphorbia*-parasitizing clade of *Hydnora* in southern Africa an apparent radiation of species is based on allopatry, extreme climatic variation, and potentially host based co-speciation. Significant work may be needed to revise the taxonomy of the Fabaceae-parasitizing clade of African *Hydnora* (excluding *H. esculenta*), where only two species are currently recognized, but where at least five formerly recognized species from east and northern Africa remain unevaluated.

## 7. Host specificity and speciation in *Orobanche minor*

C .J. THOROGOOD<sup>1</sup>, F .J. RUMSEY<sup>2</sup>, S .A. HARRIS<sup>3</sup>, S .J. HISCOCK<sup>1</sup>

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The holoparasitic angiosperm *Orobanche minor* parasitizes a taxonomically diverse range of angiosperms, and has therefore been considered to be a host-generalist. However even host-generalist parasites may show host specificity on a local or regional scale. We have examined the host specificity of *O. minor*, and its potential to act as a catalyst in driving population divergence and speciation in this parasitic plant. Divergent host ecology appears to have genetically isolated populations of *O. minor* on host species red clover (*Trifolium pratense*) and sea carrot (*Daucus carota* ssp. *gummifer*) respectively in Northern Europe. Inter-simple sequence repeat (ISSR) marker-based data provided strong evidence for genetic divergence of morphologically cryptic populations. This pattern of genetic divergence was resolved by phylogenetic analyses based on unique sequence-characterised amplified region (SCAR) markers designed from the population-specific ISSR loci. This sequence-based analysis identified host-specific clades with strong support, indicating host-driven genetic divergence has occurred in this species. We extended this analysis by sampling populations from multiple hosts across a broad geographic range across Europe. Sequence data identified an exotic host-generalist lineage and a native host-specialist lineage of *O. minor*, suggesting genetic structure in this species is defined by both host specificity and geography. These host-defined lineages appear to have hybridised where anthropogenic activity has brought them into secondary contact, which may have promoted host-shifting events. Finally, to explore the potential for a physiological basis for the observed population divergence, host-parasite interactions were examined by cultivating clovers and carrots in rhizotrons, and reciprocally inoculating them with genetic races of *O. minor* that appear to be locally adapted to these hosts. Reciprocal infection studies identified an increase in the fitness of races cultivated on their local hosts, indicating that morphologically cryptic populations in fact comprise adaptive host-specific genetic races. Using the host-generalist holoparasite *O. minor* as a model, our research demonstrates that host-driven divergence may provide a template for speciation in parasitic plants.

## 8. Proteome analysis for phylogenetic clarification in the Orobanchaceae

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The most damaging weedy root parasites belong to the Orobanchaceae, and include the *Orobanche* and *Phelipanche* genera, some of which infect important crops and causing extensive damage. In this study we used a proteomic approach to contribute to phylogenetic clarification of twelve broomrape species, based on the seed protein profile, complementing previous studies that used other approaches. Proteins were detected by Sypro Ruby staining, and the gel images were digitalized and analyzed. The 2-DE maps contained from 30 to 140 protein spots in/for the analysed species. A virtual master gel was built with spots present in all species, revealing the presence of 260 protein spots, of which only one was monomorphic while 143 were unique to a single species. Proteins were scored for the presence or absence of homologous spots, to create a binary matrix. Phylogenetic analysis was carried out using the maximum parsimony (MP) and distance methods. The trees obtained were rooted using *Cistanche phelypaea* as out-group. Both analyses showed *Orobanche* as a monophyletic group. No conclusions can be taken on *Phelipanche* due to the limited number of species included. AMOVA analysis revealed phenotypic diversity among species within genera, as well as a considerable level of variation between genera. This work represents a first attempt of using protein markers as a tool in molecular systematics of the Orobanchaceae.

## 9. Characterization of *Orobanche crenata* populations in Morocco

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In Morocco, *Orobanche crenata* is the greatest biological constraint on food legume production particularly in faba bean fields. Understanding population genetic diversity of this parasitic weed is of great importance for faba bean selection programs and breeding strategies. Our objective was to determine variability within and among six populations of *O. crenata* collected from faba bean fields in Morocco. The targeted regions are known by their importance in faba bean production and also by the presence of wide *Orobanche* infested spots. RAPD analysis was applied and twelve primers were used. RAPD profile analysis was carried out using total genomic DNA extracted from individual plants collected. Preliminary results show a high polymorphism between individuals. From 150 bands scored, 86% showed at least one polymorphism. Work is in progress on other individuals. Analysis of diversity using AMOVA will be soon finalised and results of the variation among populations and among individuals will be exposed.

## 10. Taxonomic evaluation of five *Phelipanche* species (Orobanchaceae) in Bulgaria using ISSR markers

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The genus *Phelipanche* is represented on the Balkans by seven species and one putative hybrid between *P. ramosa* (L.) Pomel and *P. lavandulacea* (Schultz) Pomel. Our current knowledge on *Phelipanche* biodiversity and distribution is based on floristic records. In this study we used ISSR markers to evaluate taxonomic diversity of the five most common for Bulgaria representatives of the *Phelipanche* genus. Specimens of *P. purpurea*, *P. arenaria*, *P. mutelii*, *P. oxyloba* and *P. ramosa* were collected from different locations in Bulgaria. Total genomic DNA was extracted from fresh flower buds and tissue and used as template for ISSR - PCR. Seven ISSR primers from the collection of the University of British Columbia (Nucleic Acid-Protein Service Unit, UBC Primer Set #9) were used to amplify polymorphic microsatellite loci. The PCR reactions were carried out in a Thermal Cycler 2720 (Applied Biosystems). To obtain uniformity in PCR reactions we used PCR master mix (Fermentas, Cat No K0171). The amplified unambiguous bands for each primer were scored manually to compile a presence/absence matrix. Dendrograms were constructed using UPGMA clustering. The results showed a specific grouping of species and sections. The grouping of specimens from sect. *Phelipanche* revealed higher than expected similarity between *P. arenaria* and *P. purpurea*, while the three species: *P. oxyloba*, *P. mutelii*, *P. ramosa* can be clearly distinguished. Most of the *P. mutelii* are clustered in a single group independently on their hosts and geographic location from which the samples were collected. Only one sample parasitizing on *Neslia paniculata* stays out of the cluster, closer to *P. ramosa* and *P. oxyloba*. The variability among *P. ramosa* specimens was higher than expected. It is independent on the hosts but depends on geographic location. The study demonstrated the high reproducibility of the results obtained by ISSR method. It will be used in the future for better understanding of biodiversity and taxonomy of *Phelipanche* species on the Balkans.

## 11. Evaluation of *Orobanche* subsect. *Glandulosae* in Bulgaria using ISSR markers

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In the Balkans, Sect. *Orobanche* is represented by 25 species, three of which are endemic for the region: *O. serbica* G.Beck et Pan., *O. esulae* Pan. and *O. pancicii* G.Beck et Petr. Samples were collected from different places in Bulgaria inhabited by four representatives of *Orobanche* subsect. *Glandulosae* (*O. alba*, *O. reticulata* subsp. *pallidiflora*, *O. serbica* and *O. pancicii*). The flower buds and tissue were used to isolate genomic DNA and run PCR reactions with five ISSR primers (University of British Columbia; Nucleic Acid-Protein Service Unit, UBC Primer Set #9). The amplified polymorphic bands were scored and used to build consequent cladogram. This confirmed the grouping of the known species. However the group of *O. alba* showed quite high diversity. The samples of *O. serbica* remain outgrouped probably because of the higher phylogenetic distance toward the other three species. Because the method is free of environment influence this approach could be used for better understanding of taxonomic relationships in *Orobanche*.

**PARASITE BIOCHEMISTRY  
AND PHYSIOLOGY**

## 12. Molecular, biochemical and histological characterization of the sucrose-degrading enzymes involved in the sink-strength of *Phelipanche ramosa*

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*Phelipanche ramosa* (syn. *Orobanche ramosa*) is an obligate parasite of major crops such as tobacco, tomato, oil seed rape and hemp. It has been known for more than two decades that the host-derived sucrose supports parasite growth. This is why sucrose metabolism is essential to promote the sink strength of the parasite. This study aimed to characterize the different sucrose-degrading enzymes including cell wall invertases (CWI), vacuolar invertases (VI), neutral/alkaline invertases (NI) and sucrose synthases (SuSy), involved in sucrose mobilization in *Phelipanche ramosa* growing on tomato roots. Tubercles displayed an equilibrated VI / NI ratio while this ratio was stronger in the growing subterranean stems due to a specific increase in VI activity. Four cDNAs, (*PR-VI1*, *PR-VI2*) encoding putative vacuolar invertases and (*PR-NI1*, *PR-NI2*) encoding putative neutral/alkaline invertases, were isolated from *P. ramosa*. Real-Time RT-PCR analysis revealed that *PR-VI1* was stronger expressed than the three others in the subterranean stems of the parasite. Moreover, after purification and sequencing of the most active vacuolar invertase in *Phelipanche*, we obtained fourteen tryptic digested peptides that matched with the putative amino acid sequence deduced from *PR-VI1* cDNA. On the other side, equal SuSy and VI activities occurred in tubercles. Using antibodies raised against faba bean SuSy, we showed that this enzyme is strongly associated to tracheids and amyloplasts contained in parenchyma cells. Two cDNAs (*PR-SUS1* and *PR-SUS2*) encoding putative SuSy were cloned and Real-Time RT-PCR analysis revealed that the expression of the two genes was differentially regulated; *PR-SUS1* was predominantly expressed in tubercles whereas *PR-SUS2* exhibited a constitutive expression. These results emphasise the key role of the vacuolar invertase *PR-VI1* in the sink strength of the developing shoots and flowering spikes of the parasite where cell expansion occurs mainly through vacuolar hexose accumulation. Nevertheless, although CWI activity was constant to a lower level in tubercles and stems, it cannot be excluded that CWI also plays a role in sucrose unloading in those organs. We may also hypothesise that SuSy is active mostly in the sink strength of tubercles where sucrose mobilization serves to the synthesis of cell wall polysaccharides in tracheids and starch in parenchyma cells.

### 13. Metabolome analysis of *Orobanche minor* seed germination for selective control of parasitic weeds

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Parasitic plants, *Striga* spp., *Orobanche* spp. and *Phelipanche* spp. cause serious damage to agriculture worldwide. A novel and effective strategy for the parasitic weed control is desired for economical and humanitarian reasons. Since the life cycle of parasitic weeds is significantly different from that of host plants, understanding of the parasite-specific biological events is important for design of selective control strategies. We focused on the germination process of parasitic weeds to find biological events specific to these species. In this study, metabolic profiling of *O. minor* seeds was conducted to reveal parasite-specific metabolic pathways during germination. Metabolic profiling with GC/TOF-MS revealed that activation of metabolism occurred only after a synthetic strigolactone GR24 treatment in seeds of *O. minor*. One of the identified metabolites, allantoin was found to decrease immediately after GR24 treatment. Acetohydroxamate (AHA), an allantoin catabolism inhibitor, reduced the germination rate of *O. minor* when the inhibitor was applied with GR24. This reduction was recovered by additional treatment with excess amounts of allantoin catabolites, such as urea and ammonium. Moreover glutamine was increased in parallel with the allantoin degradation. In *Arabidopsis thaliana* and some other plants seeds, arginine-rich storage proteins are pooled as a nitrogen source and arginine is remobilized during germination but we could not detect arginine in *O. minor* seeds. From these results, we hypothesize that allantoin is pooled in *O. minor* seeds as a nitrogen source. The inhibitory effect of AHA was only significant for *O. minor* seeds but not for *Arabidopsis* and red clover seeds. Another metabolite, gentianose was also found to decrease by GR24 treatment. Gentianose is a trisaccharide consisted of two glucoses and a fructose. The amounts of glucose and fructose significantly increased after GR24 treatment indicating these were supplied with the hydrolysis of gentianose. An inhibitor of gentianose decreased the germination rate and also the amounts of glucose and fructose. This inhibitor was also effective for *Striga gesnerioides* but not for *Arabidopsis* and red clover. These results will provide us a new target for the parasitic weed control.

#### 14. Could plant viruses move from a host plant to the parasitic weed *Phelipanche*?

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Parasitic weeds such as *Phelipanche* are a major constraint to production of many crops causing severe losses in yield quality and quantity. *Phelipanche* physically attach to their hosts through haustorial structures which connect the vascular systems of both the parasite and the host. In this study we investigated the movement of viruses from infected tobacco and tomato plants to their parasite *Phelipanche aegyptiaca* (syn. *Orobanche aegyptiaca*). Our results demonstrate for the first time that the parasite can be infected by host viruses belonging to various groups: tobacco mosaic virus (TMV), cucumber mosaic virus (CMV), potato virus Y and tomato yellow leaf curl virus (TYLCV). In order to confirm the existence of infectious CMV RNA in *P. aegyptiaca* tissues, we used *Nicotiana tabacum* and *N. glutinosa* as hosts for the parasite, exhibiting CMV systemic and local lesions (tested using *Chenopodium quinoa*) symptoms for back-inoculation. Sap-extract from *Phelipanche* tissue grown on virus-infected hosts was found to be infective on test plants. We also detected accumulation of both plus and minus RNA strands of CMV and TMV in *P. aegyptiaca* stems grown on infected tomato or tobacco, by RT-PCR. In addition, CMV particles and CMV-siRNAs accumulated to high level in *P. aegyptiaca* stems grown on infected tobacco plants. TYLCV DNA was also detected by PCR in the *Phelipanche* stems grown on tomato infected with the virus. These results indicate that CMV is not only transferred from the host to these parasites, but also replicates in the parasite tissues. We therefore conclude that *Phelipanche* spp. may serve as a reservoir for viruses in the field.

## 15. Research progresses of *Cistanche deserticola* and *Cynomorium songaricum* in western China

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*Cistanche deserticola* Y. C. Ma and *Cynomorium songaricum* Rupr. are both holoparasitic angiosperms which are widely distributed in the western desert areas of China. Both of them are extensively used in traditional Chinese medicine. *C. deserticola* parasitizes the root of *Haloxylon ammodendron* while *C. songaricum* parasitizes *Nitraria tangutorum*. In Chinese traditional medicine, their succulent stems are used as drugs. The efficacy is described as nourishing the blood, moistening the intestines, and, "locking up the kidney essence", which assures male potency in old age. The pharmacological properties of them reveal that they can improve sexual function, scavenge free radical, anti-oxidation, anti-anoxia, anti-aging, anti-fatigue, and enhance immunity. Now, many researchers focus on the biotechnology, pharmacology and cultivation of them in China. The active compounds of *C. deserticola* are mainly phenylethanoid glycosides. Those of *C. songaricum* are tannins, polysaccharide, catechin, ursolic acid, and so forth. According to the references, there are some similar characters between them: they are both obligate parasitic plants and their host plants are xerophyte. Both of them have similar embryogenesis and their seed maturation need after-ripening. Callus and suspension cell lines of *C. deserticola* have been established. We have induced callus from fleshy stems and seeds of *C. songaricum*. Researchers studied their seed germination and primary haustoria development in morphology. *C. songaricum* is our main material. Tissue culture and rapid propagation of the host of *C. songaricum* have been established. It is our objective to isolate some compounds from the root extracts of *N. tangutorum* stimulating germination or haustorium development of *C. songaricum*. Acetone extracts from roots of *N. tangutorum* were used for inducing seed germination of *C. songaricum*. The germination rate was 54%. When  $0.2 \text{ mmol.L}^{-1}$  NAA and  $0.1 \text{ mmol.L}^{-1}$  6-BA were used, the rate was 36%. Both of the plants have been cultivated successfully making for considerable economic benefits. But the low germination rate and the survival rate become the major limiting factors of the lager-scale cultivation. Therefore, more studies are needed on seed germination, haustorium formation, and the communication method between them and their hosts should be done.

## 16. Stable isotope ratios and mineral nutrient composition of *Cynomorium coccineum* and its halophytic host *Zygophyllum qatarense* in Bahrain

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*Cynomorium coccineum* L. is found in high densities along the Southern West coastline of the main island of the Kingdom of Bahrain. Their intensity decreases toward the centre of the island and they diminish along the Southern East coastline. The parasite starts its life cycle as a spherical tubercles rhizome attached to the host root. It remains under the ground until it reaches the flowering stage. During the flowering season, which ranges from December to March in Bahrain, it emerges above the ground as a fleshy red stem covered with dense flowers. This work was conducted to study the nutrient relations of *C. coccineum* and its common host in Bahrain, *Zygophyllum qatarensis* to identify the source of carbon, nitrogen and the mineral nutrients used by the parasitic plant. Dry matter <sup>13</sup>C and <sup>15</sup>N and mineral nutrients composition of four different developmental stages of the parasite and the root and the shoot of its host were evaluated. The results of the analysis revealed no significant difference between host root <sup>13</sup>C -values and the various developmental stages of the parasite. However, a significant variation was observed when host shoot <sup>13</sup>C was considered. Significant variations between host root and shoot <sup>15</sup>N -values and that of the various stages of the parasite were revealed. The holoparasite were enriched in <sup>15</sup>N-values relative to their host. These findings suggest that *Cynomorium* is a carbon root parasite but it can rely on other resources for nitrogen acquisition. The mineral nutrient status of the host/parasite pairs suggests that the parasite may rely on the host root sap for the supply of S, Fe, Zn and Mg throughout their developmental stages. Whereas, K, Ca, Mn and B might be derived from the host only during the early stages (spherical and elongated underground rhizomes). P content of the parasite resembled that of the host shoot, suggesting the possibility of remobilization of P from the shoot into the parasite tissue via the phloem. The significantly elevated level of Na in the spherical tubercles rhizomes implies its involvement in osmoregulation to facilitate water flow into the parasite and hence ensure rapid growth and development.

## 17. Effect of *Orobanche foetida* parasitism on carbohydrates and organic acid composition in faba bean

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Broomrapes (*Orobanche* spp.) are obligate parasites that infect roots of many economically important crops, causing serious losses in yields. *Orobanche foetida* Poir. is an emergent agricultural problem on faba bean in the Beja region in Tunisia. Recently, some faba bean genotypes like XBJ90.03-16-1-1 were identified as partially resistant to *O. foetida* in Tunisia. Knowing that the broomrape depends primarily on phloem sap of the parasitized plant, we carried out the analysis of the composition of soluble sugars and organic acids of the phloem exudates from adult leaves (at pod setting stage) of different faba bean genotypes (XBJ90.03-16-1-1, Giza429 and Baraca, partially resistant to *O. foetida* and Bachaar, susceptible). Analysis of host phloem sap was made in order to test the effect of *O. foetida* parasitism on carbohydrates and organic acid composition in resistant and susceptible faba bean genotypes. Faba bean genotypes were grown in association with broomrape seeds in Petri dishes. Our results showed that RFO (Raffinose Family Oligosaccharids), especially stachyose followed by sucrose were the major sugars in phloem exudates of the different genotypes. Organic acids were represented by malate and essentially citrate. The levels of carbohydrates in phloem exudates did not change significantly in response to broomrape attack for the different genotypes except for Baraca where parasitism induced a decrease in these compounds. For organic acids, only the susceptible Bachaar showed a significant increase in response to broomrape infestation. However for the other genotypes, the level of these compounds did not change significantly.

## **18. The structure of the endoparasite *Pilostyles ulei* (Apodanthaceae) in *Mimosa* hosts: vegetative body and vascular connection**

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*Pilostyles* (Apodanthaceae), endoparasitic angiosperms on Leguminosae stems (especially Mimosoideae), represent one of the most remarkable examples of plant body reduction. This study focuses on the vegetative body of *P. ulei*. From an external view, there are only tiny flowers which break out the host periderm. Internally, the flowers are connected to masses of a vegetative body. These parenchymatic masses have no storage function and are located mainly in the host phloem. From them, groups of twisted tracheary elements surrounded by parenchyma cells - called sinkers - run into the host secondary xylem. Tracheary elements were identified as tracheids, with helicoidal to scalariform thickenings. Due to their disorganized and twisted features, the parasite tracheids are not perfectly adjacent to each other and to the rest of the body. The contact zone shows wide morphological plasticity. Here we describe, for the first time, the wide range of sinker positioning in the host-parasite interface. Usually sinker parenchyma contacts the host parenchyma. Consecutive sections show that only a few parasite tracheids successfully reach host vessels. There are unique associations between parasite tracheids and host vessels, which lead to other forms of contact. Sinkers can rotate and be laterally positioned to the host vessels. Moreover, host vessel elements can head for sinkers. Sinker development causes gaps in the host tissues. The protoplast of parasite parenchyma cells that are not associated with sinkers can occupy the host cell lumina. Sinker penetration begins through host ray cells and spreads to secondary xylem cells. Parasite and host cells do not form secondary plasmodesmata. However, host wall degeneration can occur beside a parasite cell. Host cells can also be completely deteriorated or accumulate phenol compounds.

## **19. Evolution of the osmolality, proline and certain polyols contents in *Orobanche crenata* and its host *Vicia faba* subjected to water stress**

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Plants of faba bean (*Vicia faba* L.) were grown in pots under a plastic tunnel, in presence or not of seeds of *Orobanche crenata* Forsk. at a density of 600 seeds / kg of soil. The plants were first irrigated until 100 % of field capacity, and then subjected to water stress equivalent to 67 % and 33 % of field capacity, from the host bloom beginning stage to its harvest. Analyses of proline by spectrophotometer and polyols by high performance liquid chromatography (HPLC) as well as the measures of the osmolality of the cellular liquid were done for leaves and roots of the host as well as for stems of the parasite. These analyses and measures were done in pre-emergence and post-emergence phases of parasite development. The results obtained showed that inositol was present in the host and in the parasite contrary to mannitol which was identified only in *Orobanche*. These compounds as well as proline and osmolality increase significantly with water stress intensity in both the host and the parasite. Proline contents were higher in the parasite at pre-emergence stage than at the post emergence stage while we observe the opposite trends for mannitol. Osmolality measured for stems of the parasite was significantly higher than that for the host root whatever is the imposed water regime. In addition, the osmolality of the parasite seems to be high at pre-emergence phase. The implication of the measured parameters in the *Orobanche* behavior and in its water nutrition under water stress conditions will be discussed.

## **20. Contents of certain heavy metals and toxic elements in crenate broomrape (*Orobancha crenata*) and in its host (*Vicia faba*) collected from soils irrigated with wastewater**

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Crenate broomrape (*Orobancha crenata* Forsk.) is a holoparasitic angiosperm species. It causes important economic damages to different crops in Morocco and in different areas of the world. Its occurrence in plots of land irrigated with wastewater and contaminated by certain heavy metals aroused our curiosity. The analysis of irrigation waters and soils from where the plants of *O. crenata* were collected, confirmed their contamination by heavy metals. The analysis of these toxic elements in different parts of the host plant (*Vicia faba* L.) and in tubers and shoots of *O. crenata*, collected from the zone irrigated with wastewater of Fez city (Morocco), showed that these elements are also concentrated in *O. crenata*. These element contents are equivalent, and sometimes superior, to contents determined in the host organs. This suggests that there is a transfer of these metals of the host towards the parasite and that the growth and the development of *O. crenata* are not affected by these metals. The study of the effect of chromium and copper, two major heavy metals of wastewater and contaminated soils in the area of study, on seeds germination of *O. crenata* showed that no inhibitive effect was observed up to a concentration of 5 ppm. This result seems to be in agreement with field observations and might suggest a certain resistance of *O. crenata*, suggesting the need for further study and in particular the effect of these metals on different development stages of the broomrape.

## 21. Callus induction of *Cynomorium songaricum*

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*Cynomorium songaricum* Rupr., an obligate parasite, parasitizes to the root of *Nitrarias*, an extensively used herbal medicine, which is known as "suo-yang" in China. Researches have showed that compounds of *C. songaricum* include catechin, ursolic acid, oleanolic acid (triterpenoids), polysaccharides and tannides. All of them contribute to its traditional functions of reinforcing the kidney yang, replenishing vital essence and blood, and relaxing bowels. At present, wild *C. songaricum* was excessively dug to meet the market demand. This causes the exhaustion of the wild resources and their deterioration. Although cultivation of *C. songaricum* has been developed, it cannot help for its inefficiency, low success rates, and the too long growing period. In the present study, we established callus culture methods of *C. songaricum* for the first time. Succulent stem explants of *C. songaricum* were collected after anthesis from Ordos (108.7°E 39.83°N). Surface-sterilized succulent stem portions of about 1 cm<sup>3</sup> were used to initiate callus on Murashige and Skoog's medium supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D) and 1.0 mg L<sup>-1</sup> 6-benzylaminopurine (6-BA). A soft, watery callus appeared after 60 days of culturing in dark at 25°C. The induction frequency was over 70%. The primary calli were then transferred into a proliferation subculture medium supplemented with 1.5 mg L<sup>-1</sup> 6-BA. Following two bi-weekly subcultures, a mass of calli was formed. It was found that high concentrations of 6-BA in the subculture medium significantly promoted the formation of the callus. There are still some problems. First, the callus browning is remarkable because the explants are loaded with polyphenols which easily oxidizes to quinines, which reduces the explants activity. Secondly, the explants secrete bleeding sap continuously in the course of culture. Only those explants, which do not secrete the bleeding sap can form a callus. Lastly, the duration during which fresh explants can be obtained is very short, as *C. songaricum* soon senesces after emergence.

## 22. Antifungal activity of *Cuscuta reflexa*

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Integrated disease management is a non separable part of all the eco-friendly stable agricultural programs. Plant extracts have played a significant role in the inhibition of pathogens and in the improvement of crop production. *Cuscuta* is a destructive parasitic weed. The purpose of this study was to assess its significance in fungal disease management. *In vitro*, antifungal potential of *Cuscuta reflexa* Roxb. (parasitizing *Bougainvillea glabra* L.) extracts was evaluated against five different pathogenic fungi namely, *Alternaria alternata*, *Aspergillus niger*, *Fusarium solani*, *Fusarium oxysporum* and *Macrophomina phaseolina*. Different aqueous concentrations (0, 10, 20, and 30%) of *C. reflexa* were prepared. Test concentration was added in sterilized medium by food poison technique and evaluated against each fungal strain by disc method. The fungitoxicity of extract in terms of percentage inhibition of mycelial growth was calculated. Results indicated that fungal growth inhibition was directly proportional to the concentration of *C. reflexa* extract. *Cuscuta* extract exhibited significant antifungal activity against all test fungal isolates. However, extract was highly effective against *F. solani*, *F. oxysporum* and *M. phaseolina*, and was least effective against *A. niger*. It was also found that 30% (w/v) concentration was effective in reducing the mycelial growth of fungal isolates up to 73% after 6 days of incubation. Further investigations however are required to analyze the nature of antifungal compounds in *C. reflexa* and their stability.

**ECOLOGY AND POPULATION BIOLOGY  
OF PARASITIC SPECIES**

### 23. Invasion, impact and possible integrated management of the facultative hemi-parasitic weed *Rhamphicarpa fistulosa* in rain-fed lowland rice

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*Rhamphicarpa fistulosa* (Hochst.) Benth. (Orobanchaceae) is a facultative hemi-parasitic weed of tropical Africa. Infestations in rain-fed lowland rice can be severe for farmers to abandon their fields. The aim of the research was to (i) investigate whether the *Rhamphicarpa* infested area expands on a national scale, (ii) assess the potential impact on rice yield, and (iii) find clues for integrated management strategies, avoiding reliance on herbicides. To this end, a survey carried out in 1998 in Benin, was repeated in 2007. Farmer interviews and field visits were combined with pot- and on-farm field experiments including cultivars of different rice species (*Oryza sativa*, *O. glaberrima* and interspecifics). In pots, even under moderate infestation levels rice grain yields were significantly reduced compared to *Rhamphicarpa*-free controls. Among cultivars, average parasite-inflicted relative yield loss (RYL) ranged from 14 to 46% while fertilizer applications reduced RYL from 39 to 22%. Overall average parasite-inflicted RYL was 28%. Cultivar choice significantly affected *Rhamphicarpa* seed capsule number (indicative for reproduction rate) while fertilizer application significantly reduced the number of parasites. Since *Rhamphicarpa* parasitism is facultative, we also examined its performance independently. In both pots and fields host absence did not affect *Rhamphicarpa* numbers, but it significantly reduced parasite biomass and seed capsule production. In line with the 1998 survey, the majority of rice farmers (91%) in infested areas estimated *Rhamphicarpa*-inflicted yield losses to be superior to 60%. Nearly 60% of these farmers recalled *Rhamphicarpa* problems to have started between 1994 and 1998. Of the nine communes surveyed in central and north Benin, representing four departments, five had *Rhamphicarpa*-infested inland-valleys in 2007, compared to four in 1998. The newly infested commune (Ouaké) denotes the first observation of in the department of Donga, north-west of Benin. It was concluded that *Rhamphicarpa fistulosa* is a locally serious, and steadily increasing, problem in rain-fed lowland rice in Benin. We discovered genetic variation in host resistance against *Rhamphicarpa* which could be exploited for improved control. Use of the right cultivar, strengthened by increased fertilizer applications, and possibly non-host crop rotations or fallows, could mitigate *Rhamphicarpa* effects on rice yields and slow-down parasitic weed seed bank increases.

## 24. Assessing the role of abiotic factors on *Orobanche* infestation in Solaneous crops using GIS

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*Pelipanche ramosa* (syn. *Orobanche ramosa*) and *P. aegyptiaca* (syn. *O. aegyptiaca*) constitute major constraints for solaneous crops such as tobacco and tomato throughout the Mediterranean basin. The need for a broomrape site specification study is needed for the implementation of new strategies to register the broomrape species domain. Intensive surveys were conducted all over Greece during 2002-2007 in order to obtain spatial quantitative information using GIS technologies. The objectives of our research were to: a) map the dispersal and infestation level of these two noxious broomrape species throughout Greece, b) correlate bioclimatic parameters [degreedays (DD), annual humidity index (AHI)] and soil properties [soil structure (SS), pH, organic content (OC)] with the infestation level and c) develop a model under Mediterranean conditions in Greece incorporating the aforementioned parameters as well as two morphological characteristics distinct for the species [hairs on anthers (HA) and corolla length (CL)]. According to the conducted topographic surveys, 44 broomrape spots were located. In particular, *P. ramosa* / *P. aegyptiaca* parasitized processing tomato and tobacco in Northern, Central and Southern Greece but with a variation in the infestation intensity. All data were incorporated into a GIS database and distribution maps were exported demonstrating broomrape species infestation level. A sequence of statistical analyses was conducted to estimate broomrapes's occurrence. Descriptive statistics, frequencies, contingency tables and analysis of variance were performed in order to explore the distribution of values for all variables tested. Pearson Correlation Coefficient (PCC) revealed that pH, OC, AHI, HA and CL were strongly correlated to the infestation level provoked by *P. ramosa* and *P. aegyptiaca* on solanaceous crops (-0.401, 0.562, -0.345, -0.53 and 0.6 respectively). The R<sup>2</sup> statistic, based on multiple regression analysis, indicated that the fitted model explained 67.6% of the observed variability in the level of the two broomrape species and a statistically significant relationship was revealed between the variables at the 99% confidence level. Furthermore, based on discriminant analysis the broomrape populations differed in terms of infestation level with *P. ramosa* demonstrating the more severe infestation.

## 25. Population spread, host status and damage of crop plants and weed species by *Cuscuta australis* R.Br. in Johore, Malaysia

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Dodder (*Cuscuta australis* R.Br.) is a problematic weed in abandoned, derelict, open and crop areas in Malaysia. Surveys were conducted in 2008-2009 in the state of Johore, Peninsular Malaysia to assess the extent of spread and distribution of *C. australis*, and enlist its host range and damage susceptibility among crop plants and weed species. No less than 12 crop plants and 70 weed species were hosts to *C. australis* in ca. 20,000 ha of crop and non-crop land surveyed in Johore, Peninsular Malaysia. *Asystasia gangetica*, *Mikania micrantha*, and *Chromolaena odorata* were the most common hosts among weed species while the dodder was prevalent among cover crops (*Calopogonium mucunoides* and *Pueraria phaseoloides*), young tapioca, oil palm, and rubber plants. There were site- and host-mediated differences in the extent of spatio-temporal spread of dodder throughout the areas surveyed, although no significant differences were registered on the extent of spread in the three consecutive surveys at 4-monthly intervals. The area of spread range from ca. 0.36m<sup>2</sup> to 250,000 m<sup>2</sup>. Leaf disc of weed and crop plants exposed to 0.01% or more of ethanol extracts of dodder indicated that *A. gangetica*, *M. micrantha*, *Ageratum conyzoides* and *Murdannia nudiflora* were the most susceptible weed species, while *Manihot esculenta* was among the susceptible crop plants.

## 26. Taxonomic revision of Orobanchaceae in Iran

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A taxonomic revision of the Iranian species of Orobanchaceae is presented. This family comprises 3 genera in Iran, among which the most important ones are *Orobanche* (40 species), *Cistanche* (7 Species) and *Anopalon* with only one species. This work is mainly based on the study of herbarium materials deposited in main Iranian herbaria, i.e., FUHM, IRAN, TARI, TUH and author's own collection from wild populations which are deposited in the herbarium of Guilan University (GUH). In addition, the studied species were compared with type specimens and authentic materials in the herbaria: K, B, LE. During reconsidering of Orobanchaceae for the Farsi version of Flora of Iran we found a new subspecies of *Cistanche* which is described as *C. flava* (C. A. Mey.) Korsh. subsp. *bicolor* Saeidi & Shahi. Also, *Orobanche owereinii* G. Beck is reported as a new record for the flora of Iran. *O. owereinii* is an endemic element of caucasica. Morphological variation at different levels within the genera is described and compared with different concepts of its taxonomic interpretation. Instructions on examination of key characters are given. A key to these taxa, descriptions and distribution maps and a list of host plants of different species of them in Iran are provided.

## 27. Parasitic weeds, a possible threat to fruit and forest trees in Jordan

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A field search for parasitic weeds in orchards and forestry areas was carried out for the last five years in Jordan. Results showed the presence of seven parasitic genera of six plant families attacking 60 woody species of fruit and forest trees belong to 28 families and spread in different biogeographical regions in the country. Parasitic genera recorded were *Cuscuta* (4 species), *Cistanche* (3 species), *Orobanche* (4 species), *Osyris alba*, *Plicosepalus acaciae*, *Viscum cruciatum*, and *Cynomorium coccineum*. Among the most severely and economically parasitized species were *Citrus* spp., *Olea europaea*, *Vitis vinifera* and *Zizyphus spina-christi* by *Cuscuta monogyna*; *Amygdalus communis* and *Olea europaea* by *Orobanche shultzii* and *O. cernua*; *A. communis*, *Cupressus sempervirens*, *O. europaea*, and *V. vinifera* by *Osyris alba*; *Casuarina equisetifolia*, *Haloxylon persicum* and *Punica granatum* by *Cistanche* spp.; *Acacia* spp., *C. equisetifolia*, *Ficus carica*, *Melia azedarach*, *Parkinsonia aculeata*, *Ponciana gilliesii*, *Retama raetam*, *Rhus tripartite*, *Zizyphus* spp. and *Tamarix pentandra* by *Plicosepalus acaciae*; *A. communis*, *Crataegus azarolus*, *O. europaea*, *P. granatum*, *R. raetam*, and *Rhamnus palaestina* by *Viscum cruciatum* and *H. persicum* by *C. coccineum*. Many of the host species are first time reported while *O. shultzii* was unknown before to parasitize woody plants. Several host species reported were attacked by more than one parasitic genera including *O. europaea*, *V. vinifera*, *A. communis*, *H. persicum*, *R. raetam*, *T. pentandra* and *Z. spina-christi*. Results showed the high potential of these parasites to spread and infest new regions in absence of proper control measures.

## 28. Pollination biology in the genus *Hydnora*

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*Hydnora* is a genus of root holoparasites occurring from South Africa across sub-Saharan Africa to the Arabian Peninsula and Madagascar, with an apparent centre of diversity in southern Africa. In this study we investigated the pollination biology of *H. africana* parasitizing *Euphorbia gregaria*, and *H. abyssinica* parasitizing *Acacia karroo* in the Gondwana Canyon Park in southern Namibia. Both the trimerous *H. africana* flower and the tetramerous flower of *H. abyssinica* have androecial and gynoecial chambers and attract floral visitors with strong odours emitted from osmophores. We followed the floral phenology of *H. africana* over an 18 month period, and evaluated the insect imprisonment mechanism of this species with beetle addition and pollen viability assays. Shorter term field observations over a total period of 3 years were conducted for *H. abyssinica*. In addition, thermogenesis was investigated in both species by long-term respirometry and thermometry on intact flowers, coupled with short-term measurements on floral parts during selected phases of the flowering sequence. Although both species have protogynous flowers, they showed marked differences in their phenology. The appearance of flowers of *H. abyssinica* is restricted to the months of February and March, following good rains, while flowering in *H. africana* seems opportunistic with flowers present throughout the year, although very low incidence during the winter months (May - July). *Hydnora abyssinica* completes the flowering event from bud opening to pollen release within a period of 12-48 hours. In *H. africana*, bud opening is followed by a period of 2-5 days of putrid odour production, presumed stigma receptivity and insect detention, whereafter the anthers dehisce sequentially over a period of several hours to two days. In this species, insects are imprisoned during the capellate stage and allowed to escape after pollen release through changes in the inner surface of the androecial chamber. Thermogenesis in *Hydnora* is exceptionally low while respiration tracked ambient and floral temperatures (Q10: 1.9-2.7). The lack of an inverse relationship between floral and ambient temperatures suggests that *Hydnora* is not a thermoregulatory flower. In these two species, thermogenesis appears to be associated with scent production and possibly gynoecial development, rather than having a direct benefit to beetle pollinators.

## 29. Distribution and sex ratio of the holoparasite *Pilostyles ulei* Solms-Laubach (Apodanthaceae) in Serra do Cipo, Minas Gerais, Brazil

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Apodanthaceae family includes plants without chlorophyll, whose vegetative body presents extreme reduction. Being endoparasites, it became apparent only at the flowering season, when the flowers break the periderm of the host. There are three genera in this family, in which the most widespread is *Pilostyles* Guill. This genus is composed of approximately 20 species, whose center of diversity is the tropical America, being Brazil the most representative country. In spite of that, the morphological existing studies focus on low relevant regions. There are detailed studies about the anatomy of the endophytic system and the influence on the host, but the life cycle and biology of this family still unknown. Therefore, the purpose of this study is to serve as an example for the comprehension of this mysterious angiosperms' biology, bringing relevant information about the distribution of flowers in the host branches and the specialized reproductive mode of the family. At Serra do Cipo, *Pilostyles ulei* has two host species: *Mimosa maguirei* Barneby and *M. foliolosa* Benth. var. *multipinna* Barneby. First, a survey was done about hosts parasitized in the field of study. In June of 2008, up to 50 flowers of *Pilostyles ulei* randomly in each of the signed individuals, total 5.000 flowers, were collected. The sample was fixed and analyzed at the laboratory under a stereo microscope. This method enabled the understanding of the flower's distribution in the host and the Sex Ratio of the species. The number of staminate flowers in the population is much lower than the pistillate: Sex Ratio is 11.75:1.00. This result creates the possibility of a discussion about the existence of pollen limitation. If it is true that a staminate flower produces enough pollen to fertilize 11.75 pistillate flowers, probably there should be a highly efficient system of pollination. This efficiency may be result of the existence of glandular trichomes, which secrete an adhesive on the pollen grains that sticks them to the body of the pollinator. The presence of this adhesive, in addition to the existence of a highly effective pollinator, would improve the pollination, then ensuring gene flow.

### 30. Application of ISSR methods in studying broomrape (Orobanchaceae) biodiversity in South/South-Western Balkans

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Broomrape is a common name for more than 200 species from Orobanchaceae family. Several broomrape species parasitize important crops. Our current knowledge on broomrape diversity in Southern and Western Balkans is based on floristic records. Only a few studies based on modern methods have included materials from the Balkans - mainly from Greece and Croatia. Thus the local diversity and distribution of the species is still grossly under-studied using modern approaches. This not only leads to unclear status of taxonomically problematic species of Orobanchaceae and their local intraspecific taxa in Balkans, but also hampers the assessment of their relevance as actual or potential treats for agricultural. The aim of this study was to select ISSR primers that amplify polymorphic microsatellite loci that could be used in both taxonomic and population studies. One hundred ISSR primers (University of British Columbia Nucleic Acid-Protein Service Unit, UBC Primer Set #9) were tested. Specimens from five different species (*P. ramosa*, *P. mutellii*, *P. oxyloba*, *O. alba* and *O. minor*), collected from different locations were used to isolate genomic DNA. The PCR reactions were carried out in a Thermal Cycler 2720 (Applied Biosystems). To obtain uniformity in PCR reactions we used PCR master mix (Fermentas, Cat No K0171). The amplified unambiguous bands were scored manually to compile a presence/absence matrix. The UPGMA (unweighted pair-group method with arithmetical averages) clustering was used to construct dendrograms. We found that seven ISSR primers produce polymorphic bands suitable to distinguishing subspecies and populations. Another 11 primers can distinguish higher taxonomy ranks. In general the obtained results open a good opportunity to study broomrape biodiversity in Southern and South-Western Balkans.

### 31. Putting things on their heads: host age thwarts establishment of the holoparasite *Cuscuta epithymum*

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During the last century, many specific heathland species have noticeably declined in northwestern Europe, mainly due to changes in land use and habitat loss. An example of such species is *Cuscuta epithymum* or common dodder. This rare and often threatened holoparasitic plant is mainly associated with the early successional phases of dry heathland. To provide appropriate conservation interventions, there is an urgent need of empirical knowledge on the specific conditions allowing successful establishment. In this study, a 2-year field experiment was applied in order to disentangle the effects of heathland management, the degree of heathland succession and seed-density on the complex process of establishment via seeds. Establishment and reproduction of the parasite requires successful transmission through five critical stages in its life: Seedling emergence, infection of a suitable host, establishment by making a functional haustorial connection, overwintering via haustoria and finally developing to a reproductive adult. In general, recruitment after two growing seasons was relatively low with a seedling emergence from 4.8 % and an adult establishment of less than 2 % of the sown seeds. Although a higher seed-density resulted in a higher number of seedlings, seed-density did not significantly affect relative germination percentages. The management type and subsequent heath succession had no significant effect on seedling emergence. In contrast, seedling attachment to the host, establishment and growth to the adult life-stage were hampered in older heath vegetation. Establishment was most successful in turf-cut pioneer heathland, characterised by a relatively open, low and young vegetation of *Calluna vulgaris*, the main host of *C. epithymum*. The age of *C. vulgaris* proved to be the most limiting factor. Altogether, our observations stress the importance of the vegetation structure and host quality on the process of establishment of *C. epithymum*, which is positively affected by means of cyclical mowing or turf-cutting of the vegetation. Lack of any heathland management will thus seriously restrict establishment of this endangered parasite and the species' long-term persistence.

### **32. Why are rare *Orobanche* species rare?**

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In contrast to the few weedy *Orobanche* spp. most wild species are rare and are only found in limited places in the spontaneous flora. Several of them are endangered. In the present research rare *Orobanche* spp. were studied in their habitats in Romania and in Germany. Ecological factors like climate and weather, and biological features like population size and frequency, were investigated. Standardized germination tests were carried out with the result that the germination rates in some cases were very low, in other cases the radiculae ("germ tubes") stayed very short. Transmission electron microscopy showed that most seeds were empty, when the germination rates were low. During further field investigations it was found that damage by mining insect larvae was the reason why the seeds could not be filled and stayed empty or immature. Moreover, due to the short radiculae, and also due to the stimulation of suicidal germination by the roots of associated plants, which are not parasitized (trap plants), no seed bank can be built up in these niches. In summary, compared with weedy *Orobanche* spp. the rare native species have ecological and biological disadvantages, which can explain their rarity. Proposals will be presented for the support and maintenance of rare species in their natural habitats.

### 33. Eco-biological characterisation of *Orobanche cernua* and its management in tobacco (*Nicotiana tabacum*) planted in alfisols of Southern India

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Tobacco (*Nicotiana tabacum* L.) is one of the important commercial crops of India cultivated in an area of 400,000 ha producing about 700 million kg of tobacco. In Tamil Nadu, tobacco is cultivated in an area of 5,102 ha producing around 7,800 tonnes of tobacco with an average productivity of 1,463 kg ha<sup>-1</sup>. A substantial proportion of this crop is infested with *Orobanche cernua* Loeff. Due to the infestation of *Orobanche* the tobacco productivity has declined. Though glyphosate is recommended for weed control in tobacco, it is not effective in controlling *Orobanche*. Even though hand weeding is relatively cheaper, it has limited use in *Orobanche* control. Moreover, the *Orobanche* is generally seen above ground from 45-55 days after transplanting and by that time the crop would have grown sufficiently tall that intercultivation with mechanical weeders would not generally be possible. With these backgrounds, a research project has been prepared on biological characterization and effective management of *O. cernua* in tobacco planted in alfisols of Southern India. Results revealed that the *O. cernua* seeds germinated an average of 50 days after tobacco transplanting. The fresh and dry shoot weights at 15 days after emergence (DAE) were in the range of 36.45 to 41.12 and 3.213 to 6.653 g shoot<sup>-1</sup> respectively and the average weights were 39.20 and 4.410 g shoot<sup>-1</sup> respectively. Shoot production varied from 4 to 15 with an average of 9 plant<sup>-1</sup>. Flower initiation was recorded on 10 to 13 DAE of *Orobanche*. Capsule number per shoot varied from 45 to 57 with an average of 50. Capsule weight was in the range of 0.083 to 0.121 g with an average weight of 0.095 g. The number of seeds per capsule varied from 3,654 to 4,216 with an average of 3,966 seeds capsule<sup>-1</sup>. The results of management of *O. cernua* revealed that post emergence application of imazethapyr 30 g ha<sup>-1</sup> on 55 DAT or plant hole application of neem cake 200 kg ha<sup>-1</sup> on 30 DAT is effective for control of *O. cernua* in tobacco and for maintaining higher tobacco leaf yield.

## 34. Outlook on parasitic weeds of rain-fed rice in Africa

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Parasitic weeds constitute an increasingly important group of weeds in rice in sub-Saharan Africa (SSA). They are reported to cause severe yield losses, sometimes resulting in complete crop failure. Most affected are the rain-fed agro-ecosystems in the Sahel and savannah zones of Africa. These agro-ecosystems comprise roughly 71% (38% upland; 33% lowland) of the total rice area in SSA, and are accountable for approximately half the total production. Parasitic weeds particularly affect the poor farmers because of the geographic overlap with these zones and agro-ecosystems and because poor farmers also generally have little means for pest control. Parasitic weeds known to affect rice in Africa (including Madagascar) are the obligate hemiparasitic witchweeds (genus *Striga*) *Striga hermonthica* (Del.) Benth., *S. asiatica* (L.) Kuntze, *S. aspera* (Willd.) Benth., *S. forbesii* Benth. and *S. brachycalyx* Skan (in decreasing order of importance) and two facultative hemiparasitic species, *Buchnera hispida* Buch.- Ham. ex. D. Don and *Rhamphicarpa fistulosa* (Hochst.) Benth. In West Africa, *S. hermonthica* and *S. aspera* are the most important obligate parasitic weeds in rice, while *S. asiatica* dominates in rice production systems in East Africa. *Rhamphicarpa fistulosa*, the main facultative parasitic weed species, is widely observed in both western and eastern parts of the continent. *Striga hermonthica* and *S. asiatica* are primarily found in free-draining uplands. *Striga aspera* can be observed in the upland to hydromorphic continuum while *R. fistulosa* favors hydromorphic zones and unimproved lowlands. Problems with parasitic weed species have been increasing in recent years and are likely to become more important for rice in Africa. In the light of projected climate change and population growth scenarios it is necessary for priority setting of research and development to get a better idea of current infestations and future invasions and effects of these parasitic weeds in rice. Few control options against parasitic weeds are yet validated and available for rice-based cropping systems. A suitable and effective parasitic weed management strategy should be based on knowledge about the biology and ecology of a particular species. Research should be aimed at filling existing knowledge gaps by focusing on rice-parasite-environment interactions and integrated management approaches.

### 35. *Cuscuta chinensis* seed – an overview

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*Cuscuta*, commonly known as Dodder, is a rootless, leafless holoparasitic weed of family Convolvulaceae among Dicots. The crop fields infested by dodders (more than 150 species) are often abandoned by farmers because the yields are zero. In this study the seed parameters such as seed size, seed length, seeds per fruit and seed output of *C. chinensis* Lam. have been studied. In addition to this seed coat which is of non-multiplicative type is reported presently. The seeds of *Cuscuta* are small mostly but rarely intermediate in size. Eventhough they are small they do not germinate during dry conditions. The seed bank is of persistent type. Treatment with concentrated H<sub>2</sub>SO<sub>4</sub> is a must for 30 minutes, to remove dormancy. Vivipary is also observed in *C. chinensis* in the present study. Differences at interspecific and intraspecific levels observed in the seed surface of *C. chinensis* in the SEM study or micromorphological study. Chlorophyllous embryos are also observed in *C. chinensis* which is a common feature in *Cuscuta*. Based on the presence of ABA in the coiled structure which is present inside the seed is interpreted that it is not a natural embryoid. The adaptations that are present in the seed which are helpful in leading the parasitic life by the adult plant are also discussed in this overview.

### **36. Ecobiological quantification and integrated management of parasitic weed *Striga asiatica* in sugarcane (*Saccharum officinarum*) planted in alfisols of southren penninsular India**

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Sugarcane (*Saccharum officinarum* L.) is one of the most important cash crops in India and plays a pivotal role in both agricultural and industrial economy of the country. Major challenges involved in the cultivation of sugarcane to achieve better productivity are nutrient, water and weed management practices. Weed infestation is one of the most dominant constraints in sugarcane production. Especially in Western Zone of Tamil Nadu more than 50% cane is cultivated in alfisols, which infestation of *Striga asiatica* L. is also more in this type of soils. *S. asiatica* removes nutrients and extract water from the host plants causing heavy losses to agricultural crops. The effect of this parasitic weed has been so devastating, the crop yield losses of 10 to 100 per cent have been recorded, leading to complete crop failure and sometimes abandonment of land. Hence an experiment was conducted to quantify the biological characteristics of *S. asiatica* and its integrated management under alfisols of Southern Penninsular India in sugarcane. Biological characters of *S. asiatica* indicated that the seeds took on an average of 49 days for emergence after cane planting with average maximum and minimum dry weight of 0.695 g and 0.530 g plant<sup>-1</sup> at seedling stage (15 DAE) and recorded maximum of 1.746 g and minimum of 1.135 g plant<sup>-1</sup> dry weight at active vegetative growth stage (30 DAE). The tiller production varied from 4 to 6 tillers with an average of 4.6 tillers per plant. Flower initiation period of *S. asiatica* varied from 26 to 32 DAE. Capsule production capacity was very high with an average of 306 capsules plant<sup>-1</sup> and with average dry weight of 0.304 g capsule<sup>-1</sup>, with each capsule containing numerous seeds. The results of integrated management of *S. asiatica* revealed that pre-emergence application of atrazine 1.0 kg ha<sup>-1</sup> on the 3rd day after planting + hand weeding on 45 DAP + earthing up on 60 DAP combined with post-emergence spraying of 2,4-D sodium salt 5 g l<sup>-1</sup> (0.5%) + urea 20 g l<sup>-1</sup> (2%) on 90 DAP have showed effective control of *S. asiatica* and for higher productivity and profitability in sugarcane cultivation.

### 37. Study of the phytosociological affinities of the parasitic weed *Orobanche crenata* with weed communities in the major host crops in eastern Algiers

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Phytosociological affinities that may exist between the parasitic weed *Orobanche crenata* Forsk. and weed communities in fields cropped mainly with faba bean (*Vicia faba* L.) in eastern Algiers were investigated during the growing season of 2008. The method of Braun-Blanquet (1951) was followed. In total, 47 relevés were randomly sampled and data were organized in phytosociological tables to distinguish syntaxonomic units. Floristic similarities between relevés and plant species assemblages were investigated with Factorial Correspondence Analysis (FCA) and Ascending Hierarchical Clustering (AHC). The intensity of the *Orobanche* infestation was estimated using the 0-6 scale of Schmitt (1981). A total of 101 species representing 8 genera and 30 families were identified. The most representative families were Asteraceae, Poaceae, Fabaceae, Brassicaceae and Apiaceae which accounted for more than 58% of the total number of species. Hierarchical clustering of vegetation data pointed out 5 species assemblages, defined according to the dominant species, namely: (1) *Medicago hispida* & *Lolium multiflorum*, (2) *Emex spinosa* & *Oxalis cernua*, (3) *Stellaria media* & *Fumaria capreolata*, (4) *Sinapis arvensis* & *Avena sterilis*, and (5) *Polygonum aviculare*. No phytosociological relationships could be found between the parasitic weed and weed assemblages identified in the present work. However, some correlations could be established between the parasitic weed and some weed species. Hence, some weeds seem to be related to no or very low infestation with *Orobanche* and those are: *Rumex crispus*, *Cynodon dactylon*, *Polygonum aviculare*, *Lythrum junceum*, *Cyperus rotundus* and *Chenopodium album*. On the contrary, the occurrence of *Emex spinosa* correlated with high infestations with the broomrape. Further ongoing *in-vitro* and *in-planta* based experiments will confirm or reject these assumptions.

### **38. Density and frequency of *Phelipanche ramosa* in tomato fields in Marmara Region**

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Tomato production is important income source of farmers in The Marmara Region of Turkey. A study was carried out in order to determine the species, density, frequency of broomrape in tomatoes in Marmara Region in 2007. Only *Phelipanche ramosa* (syn. *Orobance ramosa*) was identified. No broomrape was detected in greenhouses in Bilecik and Yalova Provinces. Broomrape was detected in 58 %, 14 %, and 50 % of tomato fields in Bursa, Kocaeli, and Sakarya provinces respectively.

### 39. Current status of Tadla region (Morocco) infestation by parasitic weeds

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Field surveys were carried out during the 2007 season to study the current situation of infestation by parasitic plants in Tadla region, Morocco. Analysis of the data showed that only three species were found as parasitic weeds: one broomrape species (*Orobanche crenata* Forsk.) and two dodder species (*Cuscuta monogyna* Vahl. and *Cuscuta campestris* Yuncker). *C. monogyna* infested important economic fruit trees such as citrus and olive, with 10 % of orchards infested. Heavy infestations caused high yield reduction. Field observations showed that *C. monogyna* has a large host range including cultivated species (*Vitis vinifera*, *Punica granatum*, *Ficus carica*) and wild species (*Crataegus monogyna*, *Ziziphus lotus*, *Sorghum halepense*). *C. campestris* parasitized mainly forage crops such as alfalfa with 45 % of fields infested and a reduction of 50 % of dry matter. Onion was the most damaged vegetable crop by this parasite followed by tomato, pepper and others. Crenate broomrape (*Orobanche crenata*) occurred mainly in zones located at the irrigated foot hills. Faba bean and pea crops were seriously damaged by this parasitic weed and nearly 100 % of fields were infested. Due to this severe infestation, these crops are now abandoned by farmers.

#### 40. *In vivo* exploration of *Phelipanche*'s populations differential parasitism

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*Orobanche* and *Phelipanche* species (broomrapes) co-ordinate their parasitism procedure with the potential host plants in order to guarantee their survival. However, holoparasitic plant seed populations respond quite differently to the chemical stimulus originating from host plants' root system, demonstrating a certain degree of host specificity. Due to the development of new races of broomrape species capable of infecting resistant crops, population studies are needed. The main research objective was to study the differentiation among and within *Phelipanche* species during germination and tubercle formation phases. Nine and 18 batches of *Phelipanche aegyptiaca* (Pers.) Pomel (syn. *O. aegyptiaca* Pers.) and *P. ramosa* (L.) Pomel (syn. *O. ramosa* L.) seeds respectively were collected during intensive surveys conducted throughout Greece during 2002-2004. In addition, four sets of seeds were obtained from intermediate forms of the afore mentioned species (species to be determined) which were traced parasitizing tobacco crops in Central Greece. On the whole 31 seed lots were evaluated for their reaction to the stimulus exerted by the tomato hybrid Heinz 9780 F1 roots which was used as a host plant. A plastic-bag assay was applied to study broomrapes and tomato interactions. In order to study broomrape populations' variability the number of germinated seeds and formed tubercles were recorded and the data were analyzed using a non parametric test Kruskal - Wallis. The germinability of *P. aegyptiaca* seeds ranged from 5% to 100% whereas the number of tubercles did not exceed 6. Despite the fact that the germination range for *P. ramosa* seeds did not differ from the previous species, the number of formed tubercles ranged from 0 to 22. Seeds from the undetermined species also demonstrated a variable response, with germination ranging from 0% to 85%, and the rate of tubercle formation ranging from 1 to 7. A statistical significant variability was observed for germinated seeds ( $K=22.32$ ,  $p=0.0001$ ) and the number of formed tubercles ( $K=17.99$ ,  $p=0.001$ ) among species and within species respectively ( $K=338.81$ ,  $p=0.001$ ) ( $K=109.50$ ,  $p=0.001$ ). Research results indicated a strong diversification in *Phelipanche* species response to tomato, an outcome that has been confirmed by *in vivo* application of other plants used as hosts as well.

#### 41. The trophic plasticity of *Phelipanche* in Bulgaria

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New data about the natural parasitism of *Phelipanche ramosa* (L) Pomel (syn. *Orobanche ramosa*) and *P. mutellii* (Shultz) Pomel in Bulgaria are collected. The information for the hosts describes 35 species from the families Brassicaceae, Solanaceae, Fabaceae, Asteraceae, Apiaceae, Poaceae, Lamiaceae, Scrophulariaceae, Chenopodiaceae, Caryophyllaceae, Araliaceae, Euphorbiaceae, Geraniaceae and Verbenaceae. The samples are collected outside the crop fields, far from the known host crops, from different parts of the country. Some of the registered hosts are new for the country. The voucher specimens with physical connection to the hosts are deposited in the Herbarium of The Agricultural University - Plovdiv (SOA).

## 42. Parasitic flowering plants in Turkey

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Due to three existing phytogeographical regions, Euro-Siberian, Mediterranean, and Irano-Turanian regions, Turkey has a very rich flora, including over 9000 plant species. Parasitic plant species from Cuscutaceae, Loranthaceae, Orobanchaceae, Rafflesiaceae, Santalaceae, Scrophulariaceae families have been recorded in the flora of Turkey. The genus *Orobanche* comprise 35 species in Turkey and constitute the most economically important genus out of four genera of Orobanchaceae, which are *Cistanche*, *Necranthus*, *Phelypaea* and *Orobanche*. *Orobanche ramosa* L. (syn. *Phelipanche ramosa*) and *Orobanche aegyptiaca* Pers. (syn. *Phelipanche aegyptiaca*) are problems in tomato and tobacco fields and *Orobanche crenata* Forsk. in faba bean. *O. aegyptiaca* and *O. ramosa* occur in lentil fields generally as mixed populations. Three species of *Orobanche* in Turkey are endemic: *O. armena* Tzvelev, *O. hodrantha* Beck, and *O. sideana* Gilli. *Necranthus orobanchoides* Gilli is another endemic species from the Orobanchaceae family. Three *Cuscuta* species are important in agricultural crops out of 15 species and there is an endemic species, *Cuscuta obtusata* Trabut. *Cuscuta campestris* Yuncker infests about 40 plant species including many crops such as tobacco, onion, pepper, and alfalfa in Turkey. *Cuscuta approximata* Bab. has been found together with *C. campestris* in alfalfa. It infests tobacco, tomato, and pepper as well. *Cuscuta monogyna* Vahl. is determined on grapevine. *Viscum album* L., *Loranthus europaeus* Jacq. and *Arceuthobium oxycedri* (DC) Bieb. are species from Loranthaceae family. *V. album* has three subspecies attacking different tree species/genera. *L. europaeus* lives especially on Fagaceae plants. It is determined on *Quercus*, *Crataegus*, and *Castana* trees. *A. oxycedri* is found on *Juniperus* spp. *Melampyrum arvense* L. from Scrophulariaceae family, is a hemiparasite, and attaches to roots. *M. arvense* var. *arvense* infests wheat in the Inner West Anatolia while *M. arvense* var. *elatius*, an endemic species, is seen in the East Blacksea Region. *Pedicularis*, *Rhynchosorys*, and *Euphrasia* from Scrophulariaceae exist in Turkey. *Euphrasia minima* Jacq. ex DC., *Rhynchosorys odontophylla* Burbidge et Richardson and *Pedicularis olympica* Boiss. are endemic species. *Cytinus hypocistis* L. and *Pilostyles haussknechtii* Boiss. are from Rafflesiaceae family. Three genera, *Thesium*, *Comandra* and *Osyris*, are recorded from Santalaceae. *T. aureum* Jaub & Spach., *T. oreogetum* Hethredge, *T. scabriflorum* Davis and *T. bertiamii* Aznar are endemic species.

### 43. Broomrape survey in tomato fields in Samsun Turkey

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Tomato (*Lycopersicon esculentum* Mill.) has an important role in human nutrition. Samsun is one of the places where tomato is growing widely in the Blacksea region of Turkey. Tomato production has been effected from a wide range of weeds. Broomrapes are parasitic plants that attack the roots of a number of important broadleaf crops including tomatoes. This survey was conducted in order to determine the broomrape species grown in tomato fields during 2007 - 2008 growing season. The survey was conducted in 102 fields in Samsun where tomato crops are widely grown. In addition, interviews were conducted with 65 farmers in order to identify their knowledge of the parasitic weeds and their control methods against to this species. Broomrapes were found in tomato fields in central Samsun and all counties where the survey was carried out. The most abundant broomrape species parasitizing tomato fields in Samsun province was branched broomrape (*Phelipanche ramosa*, syn. *Orobanche ramosa*). The frequency of broomrape was 22.33 %, the number of broomrapes branch for a tomato plant's root was 1.14 and the average of population density was 2,32 broomrape plants/m<sup>2</sup>. Results of this study showed that farmers have been lack of knowledge on either broomrape control methods or the germination and biology.

#### 44. Distribution and ecology of two *Cuscuta* species in Belgrade urban environment

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Distribution, viability and host preferences of *Cuscuta europaea* L. and *Cuscuta epithymum* (L.) Murr, were studied to establish their ability to survive under synergistic influence of ecological factors, air pollutants and general temperate-continental climatic conditions of Belgrade. Concentrations of pollutants and microclimatic parameters relevant for this study were followed during the season. *Cuscuta* species, being widespread and locally common throughout the area, are the most competitive and most adaptive selfgrown, allochthonous parasitic annuals, growing in various ruderal habitats of urban Belgrade areas. Local adaptation of two holoparasitic plants to their host plants, varied among populations. The census of the population of the *Cuscuta* species on the territory of New Belgrade without suburbs, revealed the presence of 112 individuals of *C. europaea* and 82 of *C. epithymum* over the survey period. The greatest population density was recorded at the contours of the town, being frequently found upon hedges, waste places, hedgerows, bushy places, along the roadsides, on unmowed green areas, cemetery surroundings and similar ruderal habitats. The lowest population density, however, was observed along the central streets and bank of the river Sava. Their occurrence was extremely sporadic in the parks and squares. Of the total number of the individuals of *C. europeae*, were parasitic 26% on Urticaceae, 18% on Fabaceae, 5% on Asteraceae, 5% on Solanaceae and to a lesser extent on other families (Lamiaceae, Convolvulaceae, Chenopodiaceae, Rubiaceae, Malvaceae, Plantaginaceae, and Ranunculaceae). Of total number of individuals of *C. epithymum* were growing 32% on Fabaceae, 5% on Lamiaceae, 2% on Caryophyllaceae, and occasionally on many other host plants (*Rumex*, *Potentilla*, and *Galium*). The last species very rarely parasitizes shrubs and grasses (*Cotoneaster* and *Festuca*). *Cuscuta* has been shown to be highly expansive and competitive parasitic plant, but also markedly resistant to the disturbed or stressed urban habitats. The great occurrence on unmowed green areas in newly built city residential districts indicates to the expansiveness i.e. progressiveness of the population of the *Cuscuta* in the area studied. *Cuscuta*, described as invasive, are likely to spread into native flora or managed plant systems, develop selfsustaining populations and become dominant or disruptive to those systems.

#### 45. A survey on broomrape in tobacco fields in Samsun, Turkey

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Tobacco (*Nicotiana tabacum* L.), is a major product for economic proceeds, and it is widely growing in the Black Sea Region of Turkey. However, the yields are very low due to several constraints such as pathogens, competition with perennial weeds and parasitic weeds of the genera *Orobancha* and *Phelipanche* (broomrapes). Parasitic weeds have an important role among these factors. Therefore, this study was conducted to obtain more information about the distribution and the effect of these parasitic weed in tobacco fields. In this study, 124 tobacco fields were investigated for intensity of infestation by *Orobancha* and *Phelipanche* species. In addition, interviews were conducted with 65 farmers in order to know their knowledge of the parasitic weeds and to learn about their control methods against to these parasites. Only *Phelipanche ramosa* (L) Pomel (syn. *O. ramosa*) was found to infest tobacco at the survey areas. The frequency of broomrape infection was 16.6 %, the average number of broomrapes per tobacco plant was 0.32 and the average of population was 1.93 plants/m<sup>2</sup>. The results of this study indicated that farmers lack of knowledge about the biology of broomrape, and do not use any control method against it. It was concluded that *P. ramosa* risk tobacco production in Samsun Province.

#### 46. Broomrape as a future problem for oilseed rape production in Bulgaria

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Oilseed rape (*Brassica napus* ssp. *napus* L.) is not a traditional crop for the conditions of Bulgaria. It is described in the national literature as a biological species from the Cruciferae family without economic importance for the country. Therefore detailed investigations have not been carried out on its biology, agronomic practices, genetic variability, plant protection, etc. During recent years, however, the cultivation of oilseed rape in Bulgaria increased. The areas sown with this crop increased sharply and reached 90 000 ha in 2008. While surveying winter oilseed rape crops in the region of South-East Bulgaria during the vegetation season in 2008, broomrape attack was registered. The observations revealed that broomrape was of a branched type about 0.15 - 0.20 m high, with blue-violet florets. The literary references showed that this type corresponded to the description of the species *Phelipanche ramosa* (syn *Orobanche ramosa*), reported as a parasite on oilseed rape. We performed a vegetation trial to determine if broomrapes in oilseed rape field in Bulgaria parasitising oilseed rape plants or other weed species in the field. Sand-and-soil mixture was infested with seeds from the parasite previously collected from the field. Four winter oilseed rape hybrids were sown in pots and were grown under controlled conditions. As a result from the trial we found that oilseed rape is susceptible to *P. ramosa*. All hybrids demonstrated high degree of susceptibility to broomrape. These results reinforce the need of broomrape surveys, given the increasing acreage of oilseed rape in Bulgaria. In order to prevent spread of *Orobanche* spp. to new areas, studies on ecology, population dynamics and efficient control methods are necessary.

## 47. Parasitic plants in Tajikistan and control measures

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Tajikistan is blessed with diversity of plant species. Some of these plants as weeds cause considerable losses in the agriculture of year by year. Many weed species can make the harvesting process more difficult and some of them can also harbour serious plant diseases, such as viruses. Therefore investigation on weeds and developing control measures are one of the key topics in agriculture research in Tajikistan. Our research group has been involved studies on weeds in various crops since 2003. More than 200 weed species have been found in Tajikistan of which two of genera are parasitic: *Cuscuta* spp. and *Orobanche* spp. *Cuscuta campestris* and others occur in fields and gardens, mostly on dicots, but sometimes found also on monocots. Plants appear in April and develop fast after emergence. Whitish or greenish flowers in branch axils appear from June to October, fruiting immediately after flowering up to October. Plants produce a very high amount of seeds, which survive in the soil for at least 6 years. Parasite occurs throughout the country, for some vegetables and for potatoes of importance. *Cuscuta* transfers the phytoplasma of which's broom disease of potatoes that are found in Tajikistan. To control the parasite deep ploughing has highest importance to destroy the seeds. Crop rotation, hand weeding and cleaning the seed are very important to interrupt the propagation through the soil. Another parasitic plant is *Orobanche* (*Orobanche cumana* and other species of *Orobanche*). These are annual root parasite. Plants appear in May/June, fast growing, fresh seeds germinate only in the next spring. After germinating the seed on host roots first shoots appear 1.5-2 months later above the soil surface, with very high seed potential in the soil, on dicots and monocots, also on weeds. Blue to violet flowers appear as ears on the upper stem in June/July, fruiting in August/September, seeds can survive in soil for 10 years. Has huge financial impact in cereals, in vegetables sometimes harmful. Main control measures are deep ploughing, crop rotation and seed cleaning. Mechanical control is not much effective.

#### 48. Pathogenicity of different broomrape populations on five host plant species

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Broomrapes (*Orobanche* and *Phelipanche* spp.) are holoparasites attacking many plant species and frequently cause important yield losses to crops. An experiment was conducted to study the pathogenicity of Tunisian broomrape populations (*O. crenata*, *O. foetida* and *P. ramosa*) on five host crop species (faba bean, chickpea, lentil, pea and rapeseed). *Orobanche crenata* seeds used in the study were collected previous seasons on pea (Oc1), faba bean (Oc2), chickpea (Oc3) and *Scolymus* spp. (Oc4); *O. foetida* seeds were collected on *Calycotum* spp. (Of1), faba bean (Of2, Of3) and chickpea (Of4); and *P. ramosa* on faba bean (Or1). Host plants were grown individually in pots of 25 ml volume filled with perlite. Each pot was inoculated with 20 mg of corresponding broomrape seeds. The experiment was conducted as a completely randomised design with four replicates. Plants were grown in a growth chamber maintained at 20°C and 12 h photoperiod. Plants were removed after two months, roots were gently washed and the number of broomrape attachments per plant was counted. Results showed highly significant effect of host species and broomrape species and populations on number of attachments. The level of infection varied among the host species and broomrape populations. *O. crenata* and *O. foetida* showed the highest level of infection but with clear differences between populations and host plant species. *O. crenata* populations (Oc3 and Oc2) were the most virulent to all host species except rapeseed. *O. foetida* populations (Of3, Of4 and Of1) presented attachments on faba bean, chickpea and lentil, however no attachment on pea was observed with *O. foetida* and *P. ramosa*. Maximum attachments per plant were observed on faba bean inoculated by Of4 (17.5) and on pea inoculated by Oc3 (16.25), yet both populations were collected on chickpea. *Phelipanche ramosa* (Or1) collected on faba bean was able to infect only rapeseed, and *O. crenata* (Oc4) collected on *Scolymus* developed tubercles on pea only. *Orobanche foetida* Of1 collected on *Calycotum* was able to infect slightly faba bean and lentil. This population was collected near Tunis far from the *O. foetida* infested area on crops, which prove that even populations parasitizing weeds may attack crops and develop aggressive forms over time.

#### **49. Occurrence of mistletoe (*Tapinanthus globefeous*) on orchards in central Sudan**

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Mistletoe (*Tapinanthus globefeous* (A. Rich.) van Tieghen (Loranthaceae)) is a stem parasite which causes severe damage to many fruit trees in orchards along the Blue Nile banks in central Sudan. A survey was conducted in the year 2008 by interviewing 175 farmers in Sinar State, a major producer of fruits in central Sudan. The objectives of the study were to evaluate the distribution of mistletoe, degree of infestation, the damage caused by the parasite and the knowledge of farmers of the parasite biology and control. Of the interviewed farmers, 84% confirmed the occurrence of mistletoe in their orchards. All respondents were familiar with and knew what mistletoe looks like. About 47% of the interviewed farmers observed the occurrence of the parasite during the last 30 years, and 20% more than 8 years ago. Forty two percent of the interviewed farmers indicated that mistletoe causes great damage and reduces the productivity by 50%, while 5% believed that mistletoe causes death of infested trees. Farmers failed to control the parasite using traditional methods and some of them were forced to cut down their trees. The survey indicated that only 46% of the respondents practiced pruning regularly to control mistletoe. Future work should concentrate on the biology of the parasite to find suitable measures for its control.

## 50. *Orobanche crenata*: A genuine threat to agricultural productivity of the Nile Valley in Sudan

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Faba bean (*Vicia faba* L), lentil (*Lens culinaris* S. Medick), and chickpea (*Cicer arietinum* L.) are the most important crops in the fertile strip of the alluvial soils of the Nile valley, North of Khartoum. The total area under the crops is about 80 thousand hectares. Faba bean is planted in over 70% of the area and provides about 70% of the country's needs. *O. crenata* was first reported on faba bean in 2000/2001 in an area of about 2 ha in the Northern State. A detailed survey of the infested site (154 ha) undertaken in 2001/2002 revealed that 94 % of the area is infested and that infestations were heavy in 90 % of the farms. Yield losses varied from complete to moderate. A second survey undertaken in 2002/2003 revealed that the parasite had spread into a stretch of about 60 kilometers. A subsequent survey in 2003/2004 showed that the parasite further spread in about 160 kilometers and localized infestations were reported from eleven new sites. Three isolated infestation foci were also reported in the neighboring River Nile States. A survey conducted in 2004/2005 indicated that *O. crenata* infestation amounted to about 9% of the total area (33.6 thousand ha) under the crop in the Northern State with highest infestation in Merowe governorate (2 022 ha). In the River Nile State the parasite was reported from 28 sites and the infested area was estimated to be about 1% of the area (33 734 thousand ha) under the crop. A subsequent survey conducted in 2005/2006 revealed the presence of the parasite in 99 sites in the River Nile State and the infested area was estimated to be 4.4% of the total area under the crop. In the Northern State repeated hand-pulling, avoidance of faba bean planting in the infested sites for long periods (<5 seasons) and planting of trap crops decreased infestation considerably. However, in the River Nile State a flare up of *O. crenata* infestation developed and the area under faba bean was reduced from over 30 thousand ha in 2003/2004 to 12 thousand ha in 2008/2009.

## **HOST-PARASITE COMMUNICATION**

## 51. Strigolactones: signaling molecules with surprising activities

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Plants have evolved intricate signaling mechanisms to communicate with their environment, and their partners and competitors in which they had to evolve the right balance between giving clear enough information to their partners and keeping their enemies as much as possible uninformed. Under low phosphate conditions most land plants stimulate the formation of symbiotic associations with arbuscular mycorrhizal (AM) fungi. This symbiosis is stimulated by signaling molecules, the strigolactones, which are exuded by the plant root. The strigolactones biosynthetically originate from carotenoid cleavage and should hence be called apocarotenoids. Strigolactone production and secretion is specifically increased in plants suffering from low phosphate availability thereby actively inviting mycorrhizal colonisation. But strigolactones are also the germination stimulants of the root parasitic *Striga* and *Orobancha* spp. Upon perception of the presence of a host root through its strigolactone secretion, seeds of these parasites will germinate and attach to the host. Nutrient shortage in agricultural fields is strongly aggravating the parasitic plant problem, and strigolactones may to a large part be responsible for this effect. Finally, it was recently discovered that the strigolactones are not only rhizosphere signaling molecules but also have an endogenous, hormonal function. The strigolactones are most likely the elusive Branch Inhibiting Signal that was postulated to exist based on genetic studies. Indeed, excessively branching mutants with mutations in two carotenoid cleavage dioxygenases, CCD7 and CCD8, in pea and rice were demonstrated not to exude strigolactones. Moreover, application of the synthetic strigolactone, GR-24, to the branching mutants could restore the wildtype phenotype. In conclusion, the apocarotenoid strigolactones are a new class of plant hormones with in- and external signaling roles. The implications for the control of *Orobancha* and *Striga* will be discussed. Also the further elucidation of the biosynthetic pathway, which is still only partly resolved, and its regulation by phosphate starvation are an important challenge during the next couple of years.

## 52. Quantifying the relationship between strigolactones and *Striga hermonthica* under varying levels of nitrogen and phosphorus in rice (*Oryza sativa*)

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Strigolactone exudation as well as *Striga hermonthica* (Del) Benth. germination and attachment were studied under different levels (0%, 25%, 50% and 100%) of nitrogen (N) or phosphorus (P) or both (NP) in two cultivars of rice (cv IAC-165 & TN-1). Exudation of strigolactones increased under deficient conditions (0% & 25%) of N and P while increasing N and P dose (50% & 100%) reduced the concentration of strigolactones in the exudates. Deficiency of P (0%) proved highly active for maximum strigolactone exudation, compared with rest of treatments. Exudation of strigolactones varied among cultivars. Rice cv IAC-165 produced both 2'-epi-5-deoxystrigol and orobanchol while in TN-1 only traces of orobanchol were detected. Deficiency of N or P (0% and 25%) caused more *S. hermonthica* infection compared with their high levels (50% and 100%). A positive linear relationship was found between amount of strigolactones and *S. hermonthica* germination or attachment in cv IAC-165 while in TN-1 the relationship was quadratic. Use of N and P might cause plants to lower down biosynthesis of strigolactones which ultimately help to reduce *S. hermonthica* germination. These results warrant further research into practical application. Knowing the nutritional status of soil and manipulating N P fertilization for rice, (and other cerealcrops) may be a promising strategy to reduce the damage of this notorious weed.

### 53. Broomrape seed conditioning and response to germination stimulants in soil

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Parasitic Orobanchaceae germinate only after receiving a chemical stimulus from adjacent roots of potential host plants. Soil application of synthetic stimulants was suggested as a means to eradicate soil borne broomrape seeds, by eliciting their suicidal germination. However, we observed that a few months following the application of the synthetic stimulant Nijmegen-1 to broomrape infested soil, host plant infection by the parasite had significantly increased. These results may indicate that synergism occurs between residues of Nijmegen-1 and the natural stimulants released by the crop, leading to an increase in both germination and infection. A preparatory phase of several days, termed 'conditioning', is known to be required after imbibition, thereafter the seeds can respond to germination stimulants. In a series of germination experiments we have demonstrated that non-conditioned seeds of both *Orobancha cumana* Wallr. and *Phelipanche aegyptiaca* (Pers.) Pomel (syn. *O. aegyptiaca* Pers.) are able to germinate in response to chemical stimulation by GR24 and by Nijmegen-1 even without prior "conditioning". The lag time between stimulation and germination response of non conditioned seeds is longer than for conditioned seeds, nevertheless both conditioned and non conditioned seeds reach maximal germination rates about two weeks after the onset of imbibition. A hypothesis is put forward, suggesting that conditioning allows the imbibed seeds to overcome the stress caused by failing to receive an immediate germination stimulus. Both findings should be considered when planning the management of broomrape.

#### **54. Qualitative and quantitative differences in strigolactone exudation between *Striga* tolerant and susceptible maize cultivars**

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Strigolactones are germination stimulants of root parasitic plants, hyphal branching factors of symbiotic arbuscular mycorrhizal fungi, and a novel class of plant hormones inhibiting shoot outgrowth. We already demonstrated that plants exude mixtures of strigolactones and the amounts and composition of these mixtures vary with plant species, their growth stages, and their growth conditions including nutrient availability. In the present study, characterization of strigolactones in the root exudates from maize plants including the *Striga* susceptible cultivar Pioneer and tolerant cultivars KST94 and WH502, grown hydroponically was conducted by comparing retention times of germination stimulants on reverse phase HPLC with those of synthetic standards and by using LC/MS/MS. The susceptible cultivar mainly exuded larger amounts of 5-deoxystrigol and the tolerant cultivars exuded mainly sorgomol. 5-Deoxystrigol is more stable than sorgomol and thus susceptible cultivars might induce more germination of *Striga* seeds in the fields.

## 55. Can formononetin induce germination of parasitic weeds?

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The isoflavone formononetin (7-hydroxy-4"-methoxyisoflavone), extracted from red clover (*Trifolium pratense*), was reported to activate root colonization with arbuscular mycorrhizal (AM) fungi and is available as a commercial product in the United States (PHC®, Myconate®, USA). Since compounds belonging to the chemical class of the strigolactones are not only able to stimulate *Striga* and *Orobanche* seed germination, but also act as important plant signals for stimulating the growth of AM fungi, we consequently tested formononetin for inducing germination in parasitic weeds. In laboratory experiments, formononetin (1 mg per L and 10 mg per L) activated germination of *Phelipanche ramosa* (syn. *O. ramosa*), *P. aegyptiaca* (syn. *O. aegyptiaca*) and *S. hermonthica* to similar levels as the synthetic strigol analogue GR 24 (10 mg per L). *O. cumana* and *O. crenata* did not respond to these treatments. Formononetin was further tested in a greenhouse experiment regarding its impact on the infestation level of *P. ramosa* on tobacco (*Nicotiana tabacum*). The incidence of emerged *P. ramosa* shoots was reduced up to 50% after application of 0.5 mg per 50 ml formononetin (soil drench) compared to the positive control. The results corroborate the hypothesis that parasitic weed species with a wide host range like *P. ramosa* recognize a wider range of chemicals as germination stimulants than those parasitizing only a restricted number of host species, such as *O. cumana*. The potential of formononetin for parasitic weed control should be further investigated.

## 56. Simultaneous isolation and purification of three compounds from the root extracts of *Nitraria sibirica* by HSCCC

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*Nitraria sibirica* Pall is widely distributed in the west of China, and is well known as the host of *Cynomorium songaricum*, which is a holoparasitic plant and officially listed in the Chinese Pharmacopoeia. Little information on root extracts of *N. sibirica* was reported. The acetone extracts from roots of *N. sibirica* can induce seed germination of *C. songaricum*. The germination rate was 54%. Three compounds were isolated and purified from the extracts by high-speed counter-current chromatography (HSCCC), which is a kind of support-free, all-liquid partition chromatography. The compositions of the two-phase solvent system were selected according to the partition coefficient (K) of the target compounds. The K value was defined as the peak area of compounds in the upper phase divided by that in the lower phase measured by HPLC with C<sup>18</sup> column at 254 nm. The mobile phase was 0.4% phosphoric acid, acetonitrile and water (10:10:80, v/v/v). The flow rate was 0.9 ml min<sup>-1</sup>. The HSCCC was operated head to tail with the upper phase as stationary phase of two-phase solvent system composed of n-hexane/ethyl acetate/methanol/water (2.8:5:2.8:5, v/v/v/v). The column was first entirely filled with the upper phase at a flow rate of 9.99 ml min<sup>-1</sup>. The mobile phase was then pumped into the column at a flow rate of 2.0 ml min<sup>-1</sup>, while the apparatus was run at a speed of 850 rpm. The crude extracts (400 mg) dissolved into 20 ml mixture of the upper and lower phase solutions were loaded. The whole experiment was conducted at 25°C. The effluent was continuously monitored with a UV detector at 254 nm. The peak fractions were collected according to the elution profile. Each fraction was evaporated under reduced pressure, and then dissolved by methanol for subsequent purity analysis by HPLC. The one-step purification produced 10.6 mg S1, 9.2 mg S2 and 6.3 mg S3 with a high purity of 98.1%, 94.2% and 92.5%, respectively. The identification was carried out by ESI-MS, 1H-NMR, 13C-NMR spectra. S1 was identified as ethyl trans, trans-5-(p-octyloxyphenyl)-2,4-pentadienoate, and the structures of S2 and S3 have not been analyzed yet.

## 57. Effect of plant growth-promoting rhizobacteria on the germination of *Cuscuta campestris* Yunck.

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Beneficial free-living soil bacteria are usually referred to as plant growth-promoting rhizobacteria (PGPR), which can modulate plant growth. Effects of microorganisms on the germination and seedling growth of crops have been studied by numerous scientists, but the effects of microorganisms on seed germination and young seedlings of weed species (especially of parasitic plants) have rarely been studied. The objective of this article is to provide an overview of the ecology of PGPR-*Cuscuta campestris* Yunck. seed relationship and effects of different bacteria on seed germination of this species. The effect of several PGPRs (*Bacillus licheniformis*, *B. pumilus*, *B. amyloliquefaciens*, *B. megaterium* and *Azotobacter chroococcum*) on germination of *C. campestris* seeds was studied. Incubation of weed seeds was done with 24 h old inoculate with cell concentration of  $10^8$  ml<sup>-1</sup>. Twenty seeds of *C. campestris* were placed in Petri dishes and treated with solutions containing different bacteria. Water only was added to the control variant. The number of germinated seeds was recorded daily (germination rate), and the final percentage of germination was measured after 9 days. All the experiments were carried out in the dark in an incubator (Binder CE) at 25°C. Each experiment was conducted twice. All the data were subjected to analysis of variance and means were separated by least significant differences test. Treatments were compared with different PGPR and germination was found to vary among the bacteria tested. Contrary to our expectations, all the treatments with *Bacillus* had inhibitory effect on germination of *C. campestris* (22.88 % to 100.00 % inhibition). *B. amyloliquefaciens* and *B. megaterium* reduced the number of germinated seeds 22.88 % and 50.35 %, respectively, while in treatments with *B. licheniformis* or *B. pumilus* inhibition was 100 %.

## 58. Stimulation of *Orobanche* seed germination by *Pisum sativum* root exudates

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Various *Orobanche* and *Phelipanche* species (broomrapes) are serious weed problems and cause severe reduction on yields in many important crops. Seeds of these parasitic weeds may remain dormant in the soil for many years until germination is stimulated by the release of a chemical signal by roots of a host plant. Considering that the seed germination of parasitic plants depends on the presence of stimulating exudates produced by the roots of the host plant, an alternative approach for the management of parasitic host plants is the so called “suicidal germination”. This latter consists in the induction of seeds germination by the application of a germination stimulant to the soil, in the absence of host. The parasite seeds germinate but, in the absence of the host will die in few days, resulting in a reduction of seed bank. Much attention has therefore been focused on the isolation and identification of germination stimulants. Seedlings of pea (*Pisum sativum*) were grown in hydroponic sterile deionized water and the root exudates dissolved in the water substrate were collected and freeze dried. The resulting dry powder was extracted with ethyl acetate and the resulting extract was fractionated using a combination of different chromatographic methods. The stimulatory effect of the root exudates, the extract and the purified fraction was tested *in vitro* on seeds of *P. aegyptiaca*, *O. crenata*, *O. foetida* and *O. minor* with the aims to isolate some bioactive metabolite with potential herbicide activity. In this communication the chemical characterization of some metabolites as well as their effect on stimulation of seed germination of *Orobanche* and *Phelipanche* spp. will be discussed.

## 59. Screening of strigolactone biosynthesis inhibitor for the control of parasitic weeds

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Parasitic weeds are responsible for large-scale crop devastation all over the world. The germination of parasitic weeds is induced by recognizing the secondary metabolites, strigolactones, that are derived from carotenoid and secreted from the roots of the host crops. Strigolactones are also rhizosphere signals for the symbiotic arbuscular mycorrhizal fungi, and recently, it has been reported that strigolactones and their metabolites act as a novel hormone in shoot branching. In the proposed biosynthesis pathway, carotenoid cleavage dioxygenase 7 (CCD7) and CCD8 that catalyse carotenoid cleavage reaction and a cytochrome P450 monooxygenase are involved in strigolactones biosynthesis, but most of biosynthetic enzymes are unknown. Developing and utilizing strigolactone biosynthesis inhibitors could be successful ways for the control of parasitic weeds and the clarification of the physiological functions of strigolactone. 9-cis-Epoxycarotenoid dioxygenase (NCED) that was contained abscisic acid biosynthesis is the most characterized CCD in plants. We previously identified NCED inhibitors as well as cytochrome P450 inhibitors. To design a strigolactone biosynthesis inhibitor, we screened our chemical libraries targeted to NCED and cytochrome P450s and found some candidate compounds. They contained abamine, previously identified as NCED inhibitor with unknown side effect. Abamine inhibited the germination of parasitic weeds and, as determined by LC-MS-MS analysis, it reduced the level of 5-deoxystrigol in the roots of host plant and its exudates. So we think that abamine inhibits strigolactone biosynthesis, and by structural development of abamine we will identify specific inhibitors of strigolactone biosynthesis.

## 60. Preparation of multideuterium-labeled 5-deoxystrigol as an internal standard for quantitative analyses by LC/MS

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Strigolactones are highly active bioactive molecules that induce the germination of parasitic plants *Striga*, *Orobanche* and *Phelipanche* at the concentration of 0.1-10 nM and trigger the characteristic hyphal branching of arbuscular mycorrhizal fungi at the concentration of 0.1-10 pM. Strigolactones are biosynthesized only in small quantities in plants; therefore, the identification and quantitative analyses of strigolactones are still challenging. Although LC/MS-MS is mainly used for this purpose by comparing retention times to those of standard (natural or synthesized compounds), the limited availability of these chemicals make it difficult to determine quantities of strigolactones. Since strigolactones have been demonstrated recently to function as a shoot branching inhibitor in plants and then released in root exudates, simple and precise methods for the quantitative analyses of strigolactones are more and more required. Hence, we have simply synthesized multideuterium-labeled 5-deoxystrigol (5DS) that is thought to be a precursor of strigolactones, as an internal standard for LC/MS-MS analyses. The use of the multideuterium-labeled 5DS could facilitate estimating the changes in the level of 5DS and/or conversion of 5DS to other strigolactones upon environmental change such as phosphorus deficiency. The reaction of 7,7-dimethyl-2,3,4,5,6,7-hexahydro-1H-inden-1-one with sodium hydride and diethyl oxalate followed by treatment with ethyl bromoacetate gave the diester. Acidic hydrolysis of the diester with AcOD and 20% DCI for more than 4 hr gave the multideuterium-labeled oxo acid (ca 90% introduction at each position), which was then reduced and resulting hydroxy acid was lactonized to give a tricyclic lactone. Little deuterium atoms in multideuterium-labeled oxo acid and tricyclic lactone were replaced to hydrogen atom in water for 8 days. Formylation of the tricyclic lactone with methyl deuterioformate and subsequent reaction with bromonated furanone gave racemic multideuterium-labeled 5DS and its 2'-epimer. Little non-labeled 5DS was detected in LC/MS-MS analysis although multideuterium-labeled 5DS consists mainly of [3a,4,4,5,5,9]-hexadeutero-5DS (47%) and pentadeutero-5DS (32%). Full-scan spectra of fragment ions of non-labeled 5DS and multideuterium-labeled 5DS are clearly distinguishable, suggesting that multideuterium-labeled 5DS is used to as an internal standard in LC/MS analyses.

## 61. Actinomycetal stimulation of in vitro broomrape seed germination

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Suicidal broomrape (*Orobanche* and *Phelipanche* spp.) seeds germination provoked by soil microorganisms was studied. A collection of 75 actinomycete isolates was screened for the ability to provoke seeds germination in the absence of host plants. The broomrape seeds were treated in vitro with crude and filtered liquid culture broths of the tested isolates, and the germination was observed directly under the microscope. The investigation protocol was optimized so that the provocation tests could be of sufficient duration over a large number of seeds. The supposed provoking factor produced by the microorganisms could be characterized as an extracellular, most probably secondary metabolite. The target competence was confirmed in one of the previously reported strains, and it was observed in 13 new strains (17 % of all isolates tested), originating from broomrape infested soils, from soils where no broomrapes occurred, as well as from various other sources. The diverse origins of the microorganisms provoking in vitro germination of the parasite's seeds could mean, besides all, that such an ability was not induced or selected by the presence of broomrapes in the environment. Some of the competent actinomycetes provoked germination once and did not show this aptitude again upon repeated tests. Other strains gradually lost their ability after several transfers and tests. There were also strains with permanent capability to provoke seeds germination. The percentage of germinated seeds varied greatly - from 0.3 to 7 %, depending on the strain and the initial culture medium composition. As a rule, richer culture medium yielded more provoked seeds by one and the same actinomycete. Eight of the 19 competent strains found in our previous work and in the present investigation were taxonomically identified as *Streptomyces ambofaciens*, *S. aureocirculatus*, *S. carnosus* (2 strains), *S. fasciculatus* (2 strains), *S. griseorubens*, and *S. wilmorei*. The species diversity and the considerable ubiquity of these actinomycetes suggested that broomrape seed germination might be provoked by some quite common excretion, rather than by some exotic compound. Soil microorganisms could prove to be an important mediator or a third side in the broomrape parasite-host interactions.

## **PARASITIC WEED MANAGEMENT**

## **62. PICKIT- a decision support system for rational control of *Phelipanche aegyptiaca* in tomato**

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Broomrapes (*Orobanche* and *Phelipanche* spp.) are chlorophyll-lacking root parasites of many dicotyledonous species. They cause severe damage to vegetable and field crops worldwide. Egyptian broomrape (*P. aegyptiaca*, syn. *O. aegyptiaca*) is common throughout Israel where it parasitizes a wide range of crops. It is a devastating pest in particular in the fields of processing tomato, endangering the future existence of this crop in the country. Approximately 50 experiments, conducted during the last ten years, produced excellent results in *P. aegyptiaca* control in this crop, using the herbicides sulfosulfuron, imazapic and imazamox. The efficacy of *P. aegyptiaca* control is highly correlated with both the rates and timing of herbicide application. Furthermore, parasitism of *P. aegyptiaca* in tomato is strongly temperature (Degree Days) related. PICKIT, a decision support system (DSS) for *P. aegyptiaca* control in processing tomato, was developed based on risk assessment, on a growing degree days (GDD) model, and on a herbicide rate optimization model. The models were validated under field conditions using a minirhizotron camera. The alpha version of PICKIT was evaluated in 2008 in five separate field studies. Accordingly several model parameters have been changed and the DSS was improved. A beta version of PICKIT will be evaluated in the growing season of 2009 under commercial processing tomato field conditions.

### 63. Empowering smallholder farmers for integrated striga control in Africa

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The parasitic weed *Striga* infests vast areas of arable land in Africa causing cereal grain yield losses of between 20% and 100%, resulting in the land being abandoned. The weed affects livelihood of nearly 300 million people and results in an economic loss in excess of US\$ 1 billion annually; it is a major contributor to food insecurity and rural stagnation in Africa. A new approach, based on inherited imidazolinone-resistance (IR) to a systemic herbicide (imazapyr), is now available to African smallholder farmers to control *Striga* in maize. When IR-maize seed is coated with imazapyr and planted, *Striga* weeds attempting to parasitize the maize seedlings are destroyed, either by direct contact with the herbicide or through imbibing the herbicide from the host roots. Certified IR-maize seed production and commercialization by seed companies is targeted to *Striga* infested areas. Technology deployment activities are underway in Kenya, Uganda, Tanzania and Malawi, and it is envisaged to expand to other southern and West African countries. This technology suppresses attaching *Striga* and has direct action on *Striga* seeds reducing *Striga* seed bank in the soil. Indeed, recent *Striga* control work in western Kenya indicates the *Striga* threat can be eliminated in farmers' fields and abandoned land reclaimed for farming. Yields have been shown to increase up to four-fold under *Striga* infestation compared to *Striga* resistant varieties. Though IR-maize is planted and managed as normal farmers' usual maize/any other maize seed, care must be taken to prevent damage to intercrops by avoiding their contact with the herbicide, and hence there is need for a comprehensive stewardship program during deployment. No single control practice is applicable in all situations and therefore there is need to integrate IR-maize technology with other control options such as use of *Striga* tolerant varieties, push-pull, rotations and intercropping with legumes for optimal productivity. This paper illuminates on recent experience and strides of using IR-maize in an integrated control package for managing this damaging witchweed.

#### 64. How does *Desmodium uncinatum* control the parasitic plant *Striga*?

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In subsistence farming regions of East Africa, intercropping the fodder legume *Desmodium uncinatum* with maize is a highly effective method for control for the parasitic weed *Striga hermonthica*. It has been hypothesised that compounds present in the root exudates of *D. uncinatum* have an inhibitory effect on the *Striga* lifecycle. The aim of this study was to examine the effect of a range of compounds exuded by the *D. uncinatum* roots, including the C-glycosylated flavone isoschaftoside, on the growth of *Striga* radicles and on their ability to form haustoria. *In vitro* assays reveal that these compounds cause a significant reduction in haustoriogenesis in ecotypes of both *S. hermonthica* and *S. asiatica* in a concentration dependent manner. Assays using maize or *D. uncinatum* seedlings embedded in agar have shown that the effect of *D. uncinatum* root exudates on haustoriogenesis declines with increasing distance from the root. We discuss these results in the context of the use of *Desmodium* intercrops as a control strategy for *Striga*.

## 65. National broomrape project in Turkey

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Production of several important agricultural crops (lentil, faba bean, tomato, potato, sunflower, etc.) are threatened by broomrape (*Orobanche* and *Phelipanche* spp.) in Turkey and other regions of the world. As a result of this threat, the cultivated crop areas have been declining steadily in some countries as farmers have been abandoning production of these crops in heavily infested fields. Since the magnitude of the broomrape problem and grower complaints were increasing year by year in Turkey, a National Broomrape Project has been established. The National Project is managed by the Minister of Agriculture and is supported by TUBITAK (The Scientific and Technological Research Council of Turkey). The Project is planned for 4 years (2006-2010) and now it is currently in its third year. Approximately 40 researchers and 17 different organisations (including 6 research institutes and their general office,

9 universities and 1 agrochemical company), cooperating in an attempt to solve the problem of broomrape in tomato, potato and lentil crops. The program of this project consists of research and education studies. These studies include survey, poll, control methods and extension. Surveys are conducted in lentil, faba bean, tomato, potato, sunflower and tobacco growing areas in Turkey. Research studies include the identification of insect pests and diseases of this parasitic weed, studies on germination biology, use of natural fertilisers and several plants with allelopathic properties for broomrape control, trap cropping, determination of optimum sowing dates and crop varieties, tests on solarization and some mulch materials in greenhouse tomato, studies on biological and chemical control of broomrapes. Results of some research activities under the project are being presented in the conference. With extension activities farmers will be educated on preventive measures for not spreading seeds and on broomrape control methods. Poll studies with farmers at the beginning and the end of the project, will be done to assess success of project.

## 66. Innovations for scaling-up of *Striga* mycoherbicides application in Africa

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*Striga* spp. are presenting severe constraints to cereal and legume production in semi-arid regions of Sub-Saharan Africa. An integrated approach to *Striga* management is required for which biocontrol represents a crucial component. *Fusarium oxysporum* f. sp. *strigae* (isolates Foxy2 & PSM197) are virulent and potential biocontrol agents against *S. hermonthica*. Extensive research aiming at facilitating and enhancing their field application has been carried out since the last decade. In terms of safety, the isolates are highly host specific to the genus *Striga* only, and do not produce any mycotoxic compounds that present health risks, and therefore their use poses no threat to humans or mammals. Genetic characterisation of these isolates has shown that these isolates are similar and having unique DNA sequences that enabled them to be classified as a new *forma specialis* (f. sp. *strigae*), which could ensure their bio-safety and thus greatly improve the acceptance of their use as mycoherbicides. Massive production of inoculum of these isolates was optimized based on simple, and low cost methods using locally available agricultural by-products. For practical use, the isolates were developed into Pesta granular formulation or delivered as seed treatment technology. Both delivery technologies showed compatibility and great potential and efficacy in controlling *Striga* under both controlled and field conditions, as well as they maintained excellent shelf-life after one year of storage that would be sufficient for their use under practical conditions of storage, handling and delivery. Integration of these mycoherbicidal products (granular and seed coating) showed synergy and enhanced field efficacy with *Striga*-resistant sorghum and maize cultivars, some co-coated fungicides, and demonstrated excellent control efficacy of *Striga* and improved crop performance in West Africa. Thus, these isolates are fulfilling all necessary requirements for being potential mycoherbicide candidates for scaling up to support and enhance the existing *Striga* control measures at farm level in Sub-Saharan Africa. The developed delivery innovations which are based on simple, effective and inexpensive technologies and introduced in a manner fitting with African agriculture, will enhance the formulation of successful integrated *Striga* control methods adoptable and applicable by subsistence farmers.

## 67. Colonisation of *Striga hermonthica* and its host sorghum by the mycoherbicide *Fusarium oxysporum* f.sp. *strigae*

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The root parasitic weed, *Striga hermonthica* causes serious problems to cereal production in the tropics. *Fusarium oxysporum* f.sp. *strigae* (Foxy 2), a potential mycoherbicide has been shown to be host specific and effective in controlling *Striga*. The mutual interaction between this parasite and its host, makes the timing and extent of their colonisation by the fungus, of utmost importance. Light microscopic investigations were carried out to study the ability and extent to which Foxy 2 could colonize and survive on sorghum roots, and to trace its subsequent attack of *Striga* seedlings and shoots when applied as film-coat on sorghum seeds. Microscopic investigations of sorghum roots growing from Foxy 2 coated seeds between filter paper revealed that fungal hyphae had grown along the root surface. Hyphae colonised the cortex of sorghum roots but did not penetrate the endodermis into the central cylinder and the plants remained healthy. When *Striga* seeds germinated and attached to the sorghum roots, it was observed that 21 days after sowing, hyphae had penetrated and colonised the whole *Striga* seedling (hyaline tissue, xylem elements) including the haustoria; digesting the cells. Foxy 2 hyphae were able to destroy 95% of *Striga* seedlings underground, 26 days after sowing in root chamber experiments; proving it is aggressiveness against *Striga*. Most *Striga* plants were thus prevented from emerging, therefore reducing flowering and seed formation. For the remaining *Striga* plants which managed to emerge (pot experiments), both longitudinal and cross sections showed that masses of hyphae had clogged the vessels of the shoots and could be identified even at the top of the shoots, 2 weeks after emergence. This affected water supply and subsequently killed the *Striga* plants. On the contrary, no hyphae were observed in sorghum shoots up to 11 weeks after sowing, affirming that Foxy 2 was not able to colonise the root xylem and traverse it. These results support the suitability and appropriateness of seed treatment for the delivery and field application of the mycoherbicide against *Striga*.

## 68. Mycoherbicidal management of *Orobanche cumana*: observations from three years of field experiments

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The fungus *Fusarium oxysporum* Schlecht. f.sp. *orthoceras* (Appel & Wollenw.) Bilal was found to be an effective antagonist against all developmental stages of *Orobanche cumana* Wallr. Soil-applied in a simple granular formulation, the biocontrol agent was able to reduce the parasite's emergence by up to 90 % under controlled conditions. In a first experiment carried out under natural conditions in Israel, biocontrol efficacy was much lower compared to the pot experiments, however, excellent efficacy was achieved in subsequent field trials in Bulgaria. The in-furrow application of the fungus in three consecutive years at a rate of 17 g/m led to a reduction of the number of emerged *Orobanche* shoots from 66 % to 100 %, depending on experimental site and season. Complete *O. cumana* control was achieved even with half the dose of fungal inoculum in the year 2008. The results reveal the high potential of this biocontrol agent to be used for the management of *O. cumana*, but also confirm the variability of the biocontrol efficacy which is affected by a complex of biotic and abiotic factors. Our current and future research focuses on identifying key elements within these delicate interactions.

## 69. Influence of nutritional regimes on *Fusarium oxysporum* (Isolates Iran-502 and-507) as a biocontrol agent of Egyptian broomrape (*Phelipanche aegyptiaca*)

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Egyptian broomrape (*Phelipanche aegyptiaca* (syn. *Orobancha aegyptiaca*) is a parasitic plant that attacks a wide range of crops. *Fusarium oxysporum* is a soilborne fungus with potential as a biocontrol agent against *P. aegyptiaca*. Spore shelf life and desiccation tolerance affect pathogenicity of isolates of *F. oxysporum* and are affected by media types and fungus diets. A trial was conducted to determine the most effective nutritional regimes for enhancing conidiation, disease incitement and desiccation tolerance of *F. oxysporum* isolates. Improved sporulation for isolates 502 and 507 was obtained in a pH range of 6.5 to 7.15 and 6.0 to 7.5 respectively. C:N ratio of 15:1 was suitable for both isolates. Glycerol content of 0, 2, 5 and 7% (v/v) had no significant effects on sporulation rate of isolate 507, but considerably increased sporulation rate of isolate 502 at the contents of 2 and 5% (v/v). In a host range trial, none of the 26 tested plants showed *F. oxysporum* disease symptoms. Richard solution (RS) containing 5% (v/v) of glycerol increased disease incitement in spores of isolate 507 and completely controlled *P. aegyptiaca* whereas culturing isolate 507 in semi defined medium (SD) revealed an inverse response to the glycerol content of 5% (v/v) and in lack of glycerol led to complete control of *P. aegyptiaca*. Although in the short run adding glycerol to liquid media of RS and SD did not have any significant effect on sporulation of both isolates, in the long run it was effective in increasing desiccation tolerance in poor RS and even caused germ-tube elongation in spores which were cultured in RS with 5% (v / v) of glycerol content. The highest rate of *P. aegyptiaca* control (100% mortality) was achieved by spores of isolate 507 cultured in RS containing 5% (v/v) of glycerol and SD without glycerol. The use of RS with glycerol is more economic and effective for mass production than using SD medium.

## 70. Natural *Phytomyza orobanchia* Infestations on broomrapes in Turkey

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Natural enemies attack *Orobanche* species, and their potential role in biological control are investigated under a national project in Turkey. Little is known about broomrape's insect herbivores and pathogens in Turkey. *Phytomyza orobanchia* (Diptera: Agromyzidae), a leaf mining fly was found to be the most common insect among them. It was recorded and studied under controlled conditions in previous research in other countries. To learn more about natural infestation of *P. orobanchia* in Turkey, more than 3000 broomrape shoots were collected from fields of lentil, sunflower, tobacco, field and greenhouse tomato from 16 provinces in 2007 and 2008, and were examined individually visually and by dissecting in the laboratory. Some shoots were kept in paper bags for counting adult emergence. *P. orobanchia* was the most abundant species in lentil growing areas. *P. orobanchia* and it's parasite, *Pronotalia orobanchiae* (Hymenoptera: Eulophidae) were identified by specialists. *Phytomyza* infested shoots and non-infested shoots were compared in terms of heights, shoot diameter and number of flower/capsule.

## 71. Is *Phytomyza orobanchia* fastidious?

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Twenty one phytophagous insects from 10 families have been revealed to feed on broomrapes (*Orobanche* and *Phelipanche* spp.) under field condition in Slovakia during 2003-2008. Only, *Phytomyza orobanchia* Kalténbach (Diptera: Agromyzidae), *Chyliza extenuata* (Rossi) (Diptera: Psilidae), *Diaphora mendica* (Clerck) (Lepidoptera: Noctuidae), and moths from the genus *Celypha* (Lepidoptera: Tortricidae) caused more remarkable damage. All species, except *P. orobanchia*, are rare or polyphagous. The efficiency of *P. orobanchia* as a biological control agent was therefore further monitored under natural conditions. 18 broomrape species were reported from Slovakia. Eight of them, *Orobanche alba*, *O. caryophyllacea*, *O. elatior*, *O. flava*, *O. lutea*, *O. mayeri*, *P. ramosa*, and *O. reticulata* were examined in the field, and 30 samples were collected of each species. The seed capsules and shoots of each broomrape plant were checked for presence of *P. orobanchia* larvae or pupae. *P. orobanchia* was found throughout Slovakia following the natural broomrapes distribution, from lowlands (111-538 m a. s. l., *O. alba*, *O. elatior*, *O. lutea*, *P. ramosa*), through mountain valleys (505-875 m a. s. l., *O. flava*, *O. caryophyllacea*, *O. elatior*) up to subalpine zone (1000-1550 m a. s. l., *O. mayeri*, *O. reticulata*). Not all broomrape species were equally infested, some have not been infested at all while nearby species were infested (e.g. *O. elatior* - *O. flava* or *O. mayeri* - *O. reticulata*). No *P. orobanchia* infestation was recorded for *O. elatior* and *O. mayeri*. The ascending order of other broomrapes was: *O. flava*, *O. alba*, *O. reticulata*, *P. ramosa*, *O. caryophyllacea*, and *O. lutea*, and infestation respectively accounted for 50-100, 1-70, 20-60, 1-30, 2-10, and 1-5% of infested shoots. This may indicate the existence of host preference towards some *Orobanche* species, where *O. flava*, *O. alba* and *O. reticulata* act as preferred hosts. The identification of *Orobanche* volatiles and *Orobanche*-induced host plant volatiles, which may attract *P. orobanchia* is underway.

## 72. Research on broomrape control in tomato fields in western Turkey

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Tomato is the most produced and consumed vegetable in Turkey. It is exported fresh, dried or manufactured such as paste. One of the main obstacles in tomato production is broomrape. Two species of broomrape were recorded in tomato fields in Turkey: *Phelipanche aegyptiaca* (syn. *Orobancha aegyptiaca*) and *P. ramosa* (syn. *O. ramosa*), in general as mixed populations. Lack of very efficient control methods in tomato has caused increases in broomrape problems, especially processing control has been going on for decades in the region. Research on broomrape control has been going on for decades in the region. Experiments were laid out in tomato fields naturally infested in Canakkale Province of Turkey in 2008 including tomato varieties and transplanting time, herbicides, and organic amendments (including some processed organic materials). Herbicides were applied twice (Rimsulfuron, 12.5+12.5 g/ha; Imazapic, 0.5+0.5 g/ha; Chlorsulfuron, 1.8+1.8 or 3.6+3.6 g/ha; Glyphosate 24+24 g/ha a.i.) in the fourth and sixth week after transplanting except imazethapyr (20 g/ha), which was applied once. Except rimsulfuron, all herbicides significantly decreased the number of *Orobancha* shoots as compared to no-herbicide-check until four weeks after application. However, there was no difference at the effect of treatments on the number of broomrape shoots by six weeks after application. Phytotoxic effect of imazethapyr was reflected in yield. The other herbicides gave similar yield along with the control. In organic materials experiment, the number of broomrape shoots was significantly different between treatments and check in the early counting; but, it was not statistically different at later stages. Cover crop vetch caused the least broomrape emergence. Tomato yield was significantly different. Chicken manure caused the highest yield followed by cow manure, humic acid, olive processing waste, non-treated check, chicken manure pellets, cow manure pellets, cover crop oat, humic acid pellets, cover crop vetch, cover crop wheat. The number of emerged broomrapes and tomato yield were not affected by transplanting times (30.04.2008, 13.05.2008, and 21.05.2008) or varieties (SACHTA, ALTA, and H.2710).

### 73. Management of parasitic weeds in Eastern India

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Parasitic weeds parasitize specific host plants and deprive them of water, nutrients and assimilates. They may be root parasites (*Orobanch*e, *Striga*) or shoot parasites (*Cuscuta*). They are either hemiparasites (*Striga*) or obligate parasites (*Orobanch*e) depending on the degree of parasitism. There is no single effective method for the control of parasitic weeds. The most effective approach is the integration of different environmentally friendly control measures that are economically feasible to smallholders. Parasitic weeds can be controlled mechanically by removal, which ensures complete sawing off of parasites from branches of host, ensuring removal of entire haustorial system is the best method of controlling above ground infections of *Cuscuta*. Parasitic weeds like *Orobanch*e can be controlled by periodical removal of *Orobanch*e shoots which should be destroyed by burning before flowering or setting of seeds which reduces the menace; growing of trap crops such as Sorghum, green gram, black gram in kharif season which facilitates *Orobanch*e germination but not allowed to grow which ultimately reduces the seed load in the soil; in *Orobanch*e sick fields avoid growing of solanaceous crops or skip off growing tobacco for one or two seasons. Broomrape can be controlled by treating the seeds with 2.5% CuSo<sub>4</sub> which destroys seeds of broomrape in the infested crop seed lots. *Striga* spp are best controlled by preventing their reproduction through the direct application of 2, 4-D in cereal crops by preventing seed-setting likewise post-emergence application of glyphosate @ 0.02-0.04 kg a.i. ha<sup>-1</sup> have been successfully used in some crops, such as faba beans and sunflower. Biologically, the parasitic weeds can be controlled through the effectiveness of conidial inoculum of *Fusarium oxysporium* Schlecht. f.sp. *orthoceras* (Appel & Wollenw.) a soil-borne fungus, which was determined in pots and root chamber trials with the host-parasite association; sunflower - *O. cumana*. Conidia applied postemergence infected emerged parasite shoots before they reached their flowering stage and produced seeds. Research has also been carried out for possible application of *Fusarium* pathogens for *Striga* control.

#### 74. Integrated control of *Phelipanche ramosa* on potato in Syria

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Broomrapes are major constraints for many crops. Potato has become severely parasitized by *Phelipanche ramosa* (syn. *Orobancha ramosa*) in the last decade in Syria. *Orobancha ramosa* occurs more in potato with autumn plantings than spring planting, although it can also be more exposed to *Fusarium* wilt with fall plantings and wilted broomrape shoots can become weaker and produce less seeds. Some strains of *Fusarium oxysporum*, which were collected from different wilted *Orobancha* shoots, proved effective in reducing broomrape infestation as well as increasing *Fusarium* wilt level when inoculated into potato fields. The present study aimed at an integrated control of broomrape by enhancing herbicide application with inoculation of *Fusarium oxysporum* isolates. Two post-emergence applications of 40 g ai/ha glyphosate, 3 g ai/ha imazapic, 20 g ai/ha imazathapyr and 12.5 g ai/ha rimsulfuron were tested in combinations with 5 different *Fusarium* isolates in a replicated trial. Results indicated that herbicides and *Fusarium* isolates were generally tolerated by potato, although imazapic and imazathapyr caused significant cracking of potato tubers. Glyphosate and imazathapyr significantly reduced occurrence of broomrape by 74% and 53% respectively. All of the tested herbicides reduced the dry matter of broomrape slightly but at applied doses did not increase potato yield. Several *Fusarium oxysporum* isolates reduced total numbers of broomrape and increased wilted shoots, but these did not have an impact on potato yield. These results suggest that herbicides and *Fusarium* might have a place in broomrape management. More research is needed to develop practical recommendations for herbicide-*Fusarium* combinations which are effective in controlling broomrape, maximizing crop yield and preventing carry-over of broomrape to following crops.

## **75. Integrated management of Chinese dodder (*Cuscuta chinensis*) in lucerne (*Medicago sativa*) and in *Amaranthus viridis* - a leafy vegetable**

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*Cuscuta* species have become a serious problem especially in crops like lucerne, onion, chillies, pulses and green leafy vegetables. It is an obligate parasite that attacks stems and leaves of host plants and germinates independently without any stimulant. Later on, the emerged seedlings search for a suitable host plant and send their suckers (haustoria) and live at the expense of host plants. In Tamil Nadu, lucerne and leafy vegetables (*Amaranthus viridis*) is found to be infested sporadically with *Cuscuta chinensis*, especially in Western and North Western Agro Ecological Zones of Tamil Nadu. Use of resistant cultivars, prevention of new invasion, crop rotation, tillage and management of irrigation water are found to be helpful in reducing the menace of *C. chinensis*. In pulses, pre-emergence application of pendimethalin and fluchloralin showed promising results in the management of *C. chinensis*. Hence, field experiments were conducted to manage the parasitic weed, *C. chinensis* in lucerne and leafy vegetable with the pre and post-emergence application of pendimethalin, fluchloralin, paraquat and imazethapyr. The effect of these treatments was compared with hand removal of *C. chinensis* and an uncontrolled check. The trials were laid out in randomized block design with treatments replicated three times. Results of the field experiments revealed that in lucerne, pre-emergence application of pendimethalin 1.0 kg ha<sup>-1</sup> on 3 days after sowing or hand removal of *C. chinensis* recorded lower density and dry weight of *C. chinensis* with higher green fodder yield. On the other hand, in leafy vegetable, hand removal of *C. chinensis* (or) post-emergence directed application of paraquat at 0.80 kg ha<sup>-1</sup> on 20 days after sowing resulted in lower density and dry weight of *C. chinensis* with higher green leafy vegetable yield and better economic returns.

## 76. Suppressing *Striga hermonthica* parasitism in *Zea mays* with *Sesamum indicum* and *Glycine max* and nitrogen fertilization in Benue State, Nigeria

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Maize (*Zea mays*) ranks second after rice (*Oryza sativa*) in importance in Nigeria. *Striga hermonthica* (Del.) Benth is a noxious weed that is the most serious constraint to maize production in West Africa. Yield losses due to *S. hermonthica* in maize may reach 100% for heavily infested fields and this can be very devastating to the small scale farmers. Manual weeding is the only method to control *S. hermonthica* practiced by farmers in Benue State. This method may reduce *S. hermonthica* stands per unit area but crop yield advantages are not significant. Use of tolerant/resistant crop varieties, trap crops and intercropping as well as fertilizer application, which can reduce *S. hermonthica* damage, increase and fit easily into the cropping system of the low-input farming. However, these methods have not been clearly tested in combination (Integrated *Striga* Management = ISM) to attract the attention of farmers in Benue State and therefore adoption of these techniques is not widespread. Field trials were conducted on a naturally infested field in Benue State, Nigeria to determine the effect of intercropping and nitrogen (N) fertilization on *S. hermonthica* infestation on two varieties of maize (P.Ex.Y and 9022-13STR) intercropped with either soybean or sesame at three levels of N application (0, 60, 120 kg N ha<sup>-1</sup>). The layout was a randomized complete block design with three replications. It was observed that the height of maize was significantly (P=0.05) higher in maize alone than in the intercrops and at 120 kg N ha<sup>-1</sup> compared to 0 kg N ha<sup>-1</sup> and 60 kg N ha<sup>-1</sup>. Intercropping maize with either soybean or sesame delayed emergence, flowering and fruit maturity in *S. hermonthica*. Subsequently the severity of *S. hermonthica* infestation was also significantly (P=0.05) reduced in the intercropped plots. Maize grain yield was increased with maize/soybean intercrop but not with maize/sesame. Sesame suppressed *S. hermonthica* more than soybean but at the same time depressed maize grain yields. Maize grain yield (0.59 t/ha) was higher at 120 kg N ha<sup>-1</sup> compared with the yield at 0 kg N ha<sup>-1</sup> and 60 kg N ha<sup>-1</sup> (0.34 t/ha and 0.43 t/ha, respectively). In conclusion, the advantage of using an ISM package to reduce the severity of *S. hermonthica* infestation was successfully demonstrated to farmers where maize was intercropped with soybean because sesame which was more efficient than soybean in suppressing *S. hermonthica* also depressed maize yields.

## 77. Use of local plant substances as bioherbicides against *Striga hermonthica* in Burkina Faso

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*Striga hermonthica* (Del.) Benth., is a root parasitic weed which constitutes a major biotic constraint to cereal crops production in sub-Saharan Africa. Several methods to control striga species are currently being developed and the use of natural products would be a safe alternative approach to control. Twenty five water extracts and eight freeze dried water extracts of vegetable species were screened in bioassays to evaluate their allopathic properties to control striga seed germination. Striga seeds were conditioned in 10% water extracts or in 10% diluted lyophilisates to observe their effect to inhibit striga germination. Three doses 1%, 5% and 10% of water extracts were applied respectively on conditioned striga seeds to test their ability to induce striga germination. Aqueous extracts from five species *Thevetia neriifolia* (leaves), *Azadirachta indica* (roots), *Parkia biglobosa* (peels), *Calotropis procera* (leaves) and *Balanites aegyptiaca* (roots) reduced striga germination by 93.1% to 99.8% compared to the untreated control. Aqueous extracts from *Jatropha curcas* (leaves) and *Eucalyptus camendulensis* (roots) also significantly inhibited striga germination by leading to 75.6% and 86.3% of reduction, respectively. The lyophilisats from *Calotropis procera* (leaves), *Sclerocarya bireea* (roots) and *Thevetia neriifolia* (leaves) completely inhibited striga germination. The lyophilisate from *Balanites aegyptiaca* (roots) and *Parkia biglobosa* (peels) reduced it by 99.6% and 93.5%, respectively. Aqueous extracts of *Ceiba pentandra* (bark) and *Eucalyptus camendulensis* (leaves) induced striga germination. Water extracts (1%) from both species respectively stimulated striga germination at 39.2% and 38.9% which are similar to the control (44.6%). At the doses 5% and 10%, only the extract of *Eucalyptus camendulensis* (leaves) induced striga germination ranged from 13% to 15%. Ongoing evaluation of acetonic and methanolic extracts from these twenty five species will argue the data above and the characterization of compounds will specify the chemical nature. However, the results from this study have pointed out that metabolites produced by some of local plant species may have the potential to be used as bioherbicides to control *S. hermonthica*. Indeed, they suggest that the prolonged use of non-host plants that produce *S. hermonthica* stimulants or toxins may reduce seed bank in soil.

## 78. Managing *Striga* infestation with herbicide seed treatment in acetolactate synthase resistant grain sorghum

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Witchweeds (*Striga* spp.) are parasitic plant species that create serious threats to sustainable and economic food production in Africa, being very difficult to be managed. The objective of this study was to evaluate the efficacy of herbicide seed treatments for controlling *Striga* infestation of sorghum. Seeds of an acetolactate synthase (ALS) herbicide-tolerant sorghum hybrid were treated with two ALS-inhibiting herbicides. The sorghum genotype evaluated in these trials was a F1 hybrid produced from a cross between "ATx623" and "Tailwind". ATx623 is an ALS herbicide-susceptible genotype widely used in the tropics for hybrid seed production including production of the commercially important F1 hybrids "Hageen-Durra-1" in Sudan and "NAD-1" in Niger. Tailwind is a wild sorghum accession that was collected from a corn field near Manhattan, KS. Seed treatments included three rates of imazapyr (IMI), three rates of metsulfuron-methyl (MET), and an untreated control group. In greenhouse trials, both IMI and MET treatments provided effective control of *Striga* based on attachment and emergence. Observations at 32, 46, and 60 d after planting showed that seeds treated with the highest herbicide rates had the fewest *Striga* attachments and the greatest delay in attachment. *Striga* emergence also was reduced in sorghum plants representing the highest rates of herbicide treatments. All plants in the untreated group died at or before sorghum flowering; however, herbicide seed treatments, particularly metsulfuron, provided sufficient control of *Striga*, allowing significant production of sorghum dry matter and grain and nearly normal plant height. Field studies comparing seed treatments produced similar results and indicated that herbicides provided a significant delay in *Striga* emergence and resulted in fewer emerged *Striga* plants compared with untreated controls. These studies suggest that herbicide seed treatments may provide another tool for suppressing or delaying *Striga* infestation of sorghum.

## 79. Effect of soil solarization on broomrape in greenhouse tomato

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Severe yield losses can be observed by infestation of branched broomrape (*Phelipanche ramosa*, syn. *Orobanche ramosa*) in tomato cultivation. This study was carried out to determine the effect of solarization against *P. ramosa* in two tomato greenhouses in Adana province of Turkey between 2007 - 2008. Solarization was performed by using transparent polyethylene cover (0.025 mm thick) for a period of 8 weeks during August and September 2007 in both greenhouses. To investigate the effect of this application on broomrape, some parameters such as the number of broomrape branches and broomrape weight, and tomato yield were recorded and compared with non-solarized plots in this study. As a result, solarization treatment increased maximum soil temperature by more than 10°C (13-14°C) at 5 cm depth. The temperature reached more than 50°C (51-53 °C) for 8 weeks in the two greenhouses. Solarization plots were found statistically different from the non-solarized plots regarding investigated subjects. The number of broomrape was reduced over 95% (96-97%) and tomato yield was increased by 24-27% in solarization treatment compared with the non-solarized treatment in both greenhouses. As a result, solarization for 8 weeks reduced the amount of broomrape and increased crop yields. Based on these results, soil solarization can be recommended to control branched broomrape in tomato growing areas.

## 80. Identification of molecular markers by f-AFLP technique for the detection of *Fusarium oxysporum* strain FT2, a potential mycoherbicide of *Phelipanche ramosa*

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Broomrapes (*Orobanchae* and *Phelipanche* spp.) are considered good targets for biological control and some promising fungal pathogens have been proposed, among which a strain of *Fusarium oxysporum* Schlecht.:Fr. (strain FT2) proved to be highly virulent on *P. ramosa* (syn. *O. ramosa*) plants and was proposed as a mycoherbicide for the biological control of this weed to be applied at the soil level. In order to carefully evaluate the fate of this strain after its release into the environment, a study of persistence, risk of dispersal, effect toward non-target organisms, and movement along the soil profile was needed, mainly through its monitoring into the soil. Since tracking a biocontrol agent applied to the soil is highly difficult due to the presence of a number of microorganisms living in that environment, some of which may be very similar to the strain released, the need to develop a specific marker for tracking such agent was of utmost importance. This is even more important for strains belonging to *F. oxysporum*, a species characterized by a great biodiversity. Traditional microbiological methods for the isolation of fungi from plants and soil may be used, but molecular biology now offers techniques and methods that allow us to identify microorganisms both at species and even at the strain level. Among them, amplified fragment length polymorphism (AFLP) proved to be a powerful DNA fingerprinting technique based on total genomic restriction, ligation of primer adapters, and unselective followed by selective PCR amplification of anonymous DNA fragments from the entire genome. This technique is highly versatile for the discrimination of organisms with different genomic complexity by changing the oligo design in selective amplification. We used fluorescent AFLP (f-AFLP) to screen the genetic variability of a population of *F. oxysporum* strains compared to FT2 in order to identify a specific DNA marker to be used in the development of molecular assay for the detection of FT2 strain in the soil. A wide population of *F. oxysporum* strains isolated from different hosts was screened against the mycoherbicide strain and two specific fragments belonging to this strain were identified. The two specific fragments were isolated and characterised. Their DNA sequences were utilized for primer design. A primer pair (named FT2<sub>230</sub>F/FT2<sub>230</sub>R) proved to be strain-specific and it amplified a 232 bp DNA fragment of FT2. These primers were used to monitor the presence of the *F. oxysporum* strain in the soil. Amplicons were detected from all the soil samples artificially infected by using known amounts of FT2 inoculum, whereas none of the primer sets amplified DNA from soils not infected by FT2. The development of methods for the detection of microorganisms at a strain level could also have interesting perspectives from a commercial point of view, even for agents other than mycoherbicides. For example, it could allow recognition of commercial strains and protect them from illegal production or trading, or could be decisive in case of controversies due to supposed non-target effects, e.g.: the appearance of diseases on crop plants. As far as we are aware, we used for the first time the AFLP analysis to develop a fungal strain specific marker, showing that this technique can be a powerful tool for tracking microorganisms in the environment.

## **81. Detection of Tomato Spotted Wilt Virus and Cucumber Mosaic Virus on *Cuscuta* sp. in Denizli province of Turkey**

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Tomato spotted wilt virus (TSWV) caused epidemics in many crops during growing seasons of 2005 and 2006 in Denizli province of Turkey. Tomato, pepper and lettuce were the crops most affected by TSWV in this region. *Cuscuta* sp was observed on some pepper plants, showing TSWV like symptoms. This study was carried out to determine the presence of TSWV and some other viruses on *Cuscuta* sp. during growing seasons of 2006. 16 *Cuscuta* samples growing on 16 pepper plants were tested separately by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) with commercial antisera. For testing *Cuscuta* and pepper samples specific diagnostic kits were used to detect TSWV, Cucumber mosaic virus (CMV), Tobacco mosaic virus (TMV) and Alfalfa mosaic virus (AMV). According to the results of DAS-ELISA tests all *Cuscuta* and pepper samples were found to be infected by TSWV. Furthermore 4 out of 16 *Cuscuta* samples and 4 out of 16 pepper samples were found to be infected by CMV. No positive reactions were found by TMV and AMV neither *Cuscuta* nor pepper samples. To our knowledge it is the first time that TSWV was reported on *Cuscuta* sp. in Turkey.

## **82. Control possibilities of parasitic flowering plant *Cuscuta europea* and some perennial weeds in lucerne**

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Weeds cause yield reduction and decline in seed quality in lucerne. Intensive lucerne production requires employment of contemporary weed management including chemical techniques. The greatest damage in lucerne is caused by the parasitic flowering plant European dodder (*Cuscuta europea* L.), which causes significant lucerne yield and quality reduction, and flowering and seed maturation inhibition in seed lucerne. Apart from this species, in the Republic of Serbia lucerne and red clover are both endangered also by the species *Cuscuta trifolii* Link and *C. epythimum* Murr. European dodder does not over winter only as seed that germinates from May to September, but also vegetative manner, in the root neck and in early spring it continues its development and further propagation. As a seed contaminant, European dodder demands very expensive good clean-up before marketing, as well as seed separation from lucerne seed by existing clean-up machines. Mass occurrence of the weed in later years of lucerne growing can influence economic justification of length in lucerne field exploitation. Reduction in European dodder attack has been employed preventively, by setting lucerne seed free of European dodder seed in plants for production and marketing of lucerne seed with application of electromagnetic separators, or curative, by application of herbicides aimed predominantly for treatment of European dodder local focuses. To obtain effective weed control, all control measures such as choice of the dodder-free sown area, crop rotation, soil cultivation, pure seed material, cultivation, irrigation, herbicide use and burning of heavily infested sites must be employed integratively. With the aim of chemical control of perennial weeds, treatment can be performed in 1-3 trifoliolate leaf phases, or after the first mowing when European dodder has been controlled also with herbicides based upon active ingredients: bentazone, imazethapyr, propizamid, clethodim, diquat and fluzafop-P-butyl. In European dodder control, highly successfully used has also been herbicide trifluralin. It proved highly efficient if used between January 15 and April 30, before shoot emergence of this weed.

### 83. Research on broomrape control in potato in Bozdag (Odemis), Turkey

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Potato is an important summer crop in Bozdag, a site with 1200 m of altitude in the Odemis District, Turkey. Two broomrape species (*Phelipanche ramosa* (syn. *Orobanche ramosa*) and *P. aegyptiaca* (syn. *O. aegyptiaca*) has been seen for ten years in Bozdag in spite of no broomrape infestation in autumn and spring potato cropping in the lowlands of Odemis. Field studies were carried out to control broomrape by using organic amendments (humic acid pellets at 2.5 t/ha, chicken manure pellets at 2.5 t/ha, cow manure pellets at 2.5 t/ha, humic acid at 0.3 kg/ha, chicken manure at 20 t/ha and cow manure at 30 t/ha) or herbicides (rimsulfuron at 12.5+12.5 g/ha, imazapic at 2.5+2.5 g/ha, imazapic at 5 g/ha, glyphosate at 12+12 g/ha, glyphosate at 12 g/ha, and glyphosate at 24+24 g/ha) in 2007 and 2008. Experiments were in randomized complete block design with four replications and repeated twice. Emergence and dry weight of broomrape were measured to assess the effect of treatments. Data of two years were pooled. The number of emerged broomrapes was 44 and 22 per m<sup>2</sup> in organic amendments and herbicide experiments, respectively. Chicken manure 76.5 %, humic acid 60.2 %, humic acid pellets 57.7 % and the remaining amendment treatments led to less than 50.0 % reduction in broomrape infection. Glyphosate (24+24 g/ha) 77.4 %, glyphosate (12 g/ha) 76.9 %, rimsulfuron (12.5+12.5 g/ha) 59.9 % and the remaining herbicide applications led to less than 50.0 % decreased orobanche.

## 84. Possibilities for broomrape control in tomato fields

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The infestation of broomrape (*Orobanche* and *Phelipanche* spp.) in economically important crops such as tobacco, tomatoes or sunflowers results in great losses for agriculture. The effect of soil-applied herbicides or herbicide combinations on weeds and growth of the crop has been reported. Field trials were carried out on a sandy loam soil (o.m. 1.07%, sand 73.7%, pH 6.4) at the Plant Protection Institute, Kostinbrod during 2007-2008. The present study deals with the efficacy of herbicides for broomrape control in fields of tomato, cv Ideal. The experiment was laid down in a randomized complete block design with three replications. The size of the plots were 3 m<sup>2</sup> with the following variants: Check; dazomet 4080 g/ha (Basamid granulate) / oxyfluorfen 416 g/ha (Goal 2G); metam-sodium 5880 g/ha (Nemasol 510) / trifluralin 1250 g/ha (Trifluralin 240); dazomet 4080 g/ha (Basamid granulate) / oxyfluorfen 416 g/ha (Goal 2G) / glyphosate 28 g/ha and 42 g/ha (Roundup); metam-sodium 5880 g/ha (Nemasol 510) / trifluralin 1250 g/ha (Trifluralin 240) / potassium salt of malein-hydrazid 23 g/ha and 32 g/ha (Malein-Hydrazid). Broomrape control was assessed on the 50th and 65th days after treatment and at the end of the vegetation. In order to establish the effect of herbicides on tomato plants, yield and its quality, all variants were maintained clear of weeds during the whole vegetation period. The results of this work are: 1. The herbicides metam-sodium / trifluralin / malein-hydrazid and metam-sodium / trifluralin reduced the density of broomrape by 85.9% and 76.4%, respectively. 2. There was no phytotoxicity on the leaves of the tomato plants. 3. The herbicides have favorable effects on the yield quantity. The relative yield was highest for metam-sodium / trifluralin / malein-hydrazid, followed by metam-sodium / trifluralin, while the lowest yield was obtained for dazomet / oxyfluorfen.

## 85. Evaluation of cover crops for decreasing the infestation of Egyptian broomrape (*Pelypanche aegyptiaca*)

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Parasitic angiosperms of the genera *Orobanche* and *Phelipanche* are a major constraint to agriculture in Africa, Southern Europe and Asia inflicting devastating losses to farmers. Non chemical economical methods such as using cover crops could be an effective way to control these parasitic weeds. Allelochemicals are present in almost all plants and in many tissues: leaves, stems, flowers, fruits, seeds and roots. Growing cover crops with allelopathic characteristics might be an alternative method to control Egyptian broomrape (*Phelipanche aegyptiaca* (Pers.) Pomel (syn. *O. aegyptiaca* Pers.). An experiment was conducted to compare effects of different crops on the germination rate of *P. aegyptiaca* seeds. Cotton, sesame, flax, maize, sunflower, castor, soy bean and sugar beet were grown in 2 kg pots containing sterile soil, infested with 0.9 g of *P. aegyptiaca* seeds. Control pots contained only 0.9 g of *P. aegyptiaca* seeds with no crop seeds. After 75 days plants were incorporated into the soil and tomato seedlings were planted in the pots. Eighty days later the experiment was terminated by determining the number and dry weight of the *P. aegyptiaca* plants. Results showed a significant reduction in *P. aegyptiaca* shoot number in pots which formerly contained cotton (cotton is a non-host which roots secrete Strigol a germination inducer of broomrape seeds). Comparison between means of tomato dry weight showed an advantage of planting flax before tomato and a disadvantage of planting maize and sunflower.

## 86. Environmental factors on disease incitement of *Fusarium oxysporum* attacking Egyptian broomrape (*Phelipanche aegyptiaca*)

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Egyptian broomrape (*Phelipanche aegyptiaca* (syn. *Orobanche aegyptiaca*) is an obligate root parasite which causes severe losses in crops in the Middle East. Biological control, especially using fungal antagonists of parasitic weeds, appears to be a promising tool for broomrape control. In the present research the environmental factors enhancing sporulation, spore germinability of *Fusarium oxysporum* isolates (Iran-502 & 507) and effective spore concentration for biocontrol of Egyptian broomrape, were studied. Sporulation rate (SR), spore germination rate (GR) and spore germ-tube (GT) elongation were examined under two temperature regimes (15<sup>0</sup>C and 20<sup>0</sup>C) and three illumination treatments (darkness, white and UV light). Results showed that at 15<sup>0</sup>C SR, GR and GT length of isolate 507 increased significantly. Darkness relative to the other light conditions, increased SR and GR as well as GT length. Temperature of 20<sup>0</sup>C effectively increased SR while GR was increased at 15<sup>0</sup>C. The length of GT was not significantly different at the two temperatures. Among light treatments, white light induced the highest SR, GR and GT length. The highest mortality rate of broomrape was caused by spores cultured in Richard Solution (RS) containing 5% (v/v) of glycerol. Correspondingly isolates were prepared in RS with 5% (v/v) of glycerol content in 4 concentrations 10<sup>6</sup>, 5x10<sup>6</sup>, 10<sup>7</sup>, 5x10<sup>7</sup> spores ml<sup>-1</sup>. Suspensions mentioned were used to inoculate tomato seedlings which had been planted two weeks before in the 2 kg pots (containing 1g of sterilized Egyptian broomrape seeds. Results demonstrated that in both isolates the high spore concentration of 5x10<sup>7</sup> ml<sup>-1</sup> caused a significant decrease in the mean length of broomrapes. Mean dry weight (MDW) of tomato seedlings and the number of broomrape seedlings showed no significant difference with increasing spore concentration of both isolates. Spore concentration of 5x10<sup>7</sup> ml<sup>-1</sup> from isolate 502 significantly decreased MDW of broomrape relative to other concentrations and the control treatment. Concentration of 5x10<sup>7</sup> spore ml<sup>-1</sup> in both isolates caused a significant decrease of mean capsule number in broomrape relative to the non treated control.

## 87. Effect of plant residues and exudates on broomrape germination on tomatoes

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Attempts to develop alternative methods in order to solve problems caused by intensive pesticide usage have been made in Turkey as well as worldwide. Broomrapes cause high crop losses in tomato production, which is the most produced and consumed vegetable in Turkey. In this study, the effect of plant residues and exudates on *Phelipanche ramosa* (syn. *Orobancha ramosa*) has been researched in greenhouse and laboratory experiments with five replications each. In a first experiment, wheat, barley, rye, rice, and vetch were incorporated into the soil in pots at their stem elongation growth stages and were incubated at 25°C in the dark for two months. Then 200 mg of broomrape seeds were added to each pot and two tomato seedlings were transplanted. The number of broomrape shoots was 3.2 per pot in control pots, which were filled with sterilized soil and not added any plant residue. No broomrape emergence was observed in residue treatments except for rye incorporated ones, in which the number of broomrape was 0.2. In a second experiment, exudates of wheat, barley, rye, rice, and vetch were obtained keeping 20 seedlings in 100 ml water for two days. In the pot experiment with exudates 25.4 broomrape shoots emerged. No broomrape emergence was observed in barley or rye exudate treatments; 0.2 broomrape shoots emerged under wheat and vetch applications. In a third experiment, effect of exudates on broomrape germination was studied in Petri dishes. Filter papers at 25 mm in diameter were placed in Petri dishes. 10 mg broomrape seeds were sprinkled on filter paper. Then, 2.5 ml plant extract and 2.5 ml GA<sub>3</sub> were added to each Petri dish. The number of germinated broomrape seeds was 78.4 at no-exudate-applied check. Exudates did not inhibit germination but reduced broomrape hypocotyls, which were 9.6, 1.2, 0.8, 0.6, 0.6 for rice, rye, vetch, wheat, and barley, respectively. These findings suggest that none of the plants (either residue or exudates) can control broomrape, but those plants might be used as cover crops or rotational crops to reduce broomrape infestation in the field.

## **88. Eastern dodder (*Cuscuta monogyna*) control by glyphosate in citrus and olive orchards**

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Eastern dodder (*Cuscuta monogyna* Vahl.) is a serious parasitic weed causing substantial yield losses in fruit trees such as citrus and olive. An experiment was conducted to evaluate the efficacy of glyphosate on this parasite. Glyphosate was tested at rates of 0, 50, 75, 100, 150 and 200 mg/L and sprayed on citrus (Lemon and Clementine) and olive. Results showed that a weed control of 95 % was achieved on clementine with glyphosate at rates higher than 75 mg/L but not on lemon and olive. This suggests that glyphosate absorption by Clementine leaves was more important than by lemon and olive leaves. Clementine has important foliar surface and leaves are less thick compared to those of lemon and olive. In addition, a huge infestation of trees by eastern dodder (canopy entirely covered by the weed) would be another clue of the low efficacy obtained on lemon and olive. No trees injury symptoms were observed at all rates tested of glyphosate.

## 89. Chemical control of dodder in alfalfa in conditions of Romania

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Dodders are considered the most dangerous parasite quarantine weeds in the majority of countries in South and Center of Europe, also in hot zones of South Asia, Africa, America and even in Australia. In Romania the most spread dodder species is *Cuscuta campestris* Yunck which infects alfalfa, trefoil, esparcet, sun-flower crops, vegetables and even some species of weeds from dicotyledoneus group. In Romania the first studies for *C. campestris* control have been made between 1965-1970 using herbicides based on DNOC, DNBP, diquat, paraquat and dinoseb acetat. Recently, we have conducted an experiment based on imazethapyr. Experiment has been placed by random blocks in four replications with plots of 25 m<sup>2</sup>. To ensure a heavy infection with dodder, alfalfa seed has been used infected with dodder. Imazethapyr (Pivot 100 LC) has been applied at rates 75 g/ha, 100 g/ha, and 200 g/ha when alfalfa plants had 10 - 15 cm high which were already parasitated with dodder filaments. The best results in control of dodder have been obtained applying 100 and 200 g/ha of imazethapyr with a control of 98 - 99 %. Quantity of green alfalfa was 8350 - 8400 hg/ha while the variant treated with bentazon the yeiled of green alfalfa was only 592 kg/ha. *Cuscuta* spp. infects in Romania other species such as sunflower, *Abutilon theophrasti*, and *Polygonum persicaria*.

## 90. Effect of olive wastewater on germination and early growth stages of *Orobanche crenata*

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In Morocco, food legumes cover 320 to 400 thousand ha, of which 40 to 50 % are faba beans. *Orobanche* infests more than 37 % of faba bean areas. Olive wastewater (OWW), which is a by product of the olive oil production process, affected *Orobanche crenata* infecting faba beans in previous pot experiments. The objective of this work was to investigate the effectiveness of OWW on the germination and early growth stages of *O. crenata*. Two types of experiments were conducted; (I) Test of *O. crenata* seed germination in Petri dishes in presence or absence of OWW and (II) Test of roots room -which consisted of a glass box filled with sand, allowing one to see *Orobanche* seed germination and development of tubers and buds on the host roots- with the presence of 5, 10 or 15 ml of OWW. OWW reduced the percentage of germination in Petri dishes. The reduction was even more significant as the seed remained longer in contact with the olive wastewater. The percentage of germination was 59, 13 and 5 %, comparing to the check, for 5, 8 and 13 days, respectively. In the roots room, the presence of the OWW induced the reduction of 70 to 95 % of the seed germination, 24 to 46 % of the attachment to the host radicals, 89 to 95 % of tubers and 99 % of buds depending on application rate. These data suggest that OWW application could significantly reduce the orobanche seedbank .

## 91. Intercropping with fenugreek reduce *Orobanche foetida* infection of two faba bean cultivars

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Infection with the obligate parasitic weed fetid broomrape (*Orobanche foetida* Poir.) causes considerable yield losses in faba bean (*Vicia faba* L.) and has become an important constraint to this crop cultivation in Tunisia. In order to study the effect of intercropping with fenugreek (*Trigonella foenum-graecum* L.) of two small seeded faba bean genotypes (cv. Badi, susceptible to *O. foetida* and cv. Najeh, partially resistant) on *O. foetida* infection, a trial was carried out during the cropping season 2007/08 in a field heavily and naturally infested by *O. foetida* at the Agriculture Experimental Unit of Oued Beja (36°43'N; 9°13'E) in Tunisia. Crops were sown the last week of December on five-row plots of 4 m length according a complete randomized block design with four replicates. The two faba bean cultivars were grown as monocrop and intercropped with fenugreek (Tunisian landrace) in 50% replacement model. Number of broomrapes, both emerged shoots and subterranean tubercles per faba bean plant were recorded the first week of May on five random plants from each plot. Level of infection varied among the two studied cultivars; cv. Badi was much more infected than cv. Najeh, with a final number of total broomrapes per plant of 14.25 and 3.5, respectively, observed in monocrop treatment. In addition, results showed a significant effect ( $p < 0.05$ ) of intercropping fenugreek with faba bean cultivars on *O. foetida* infection and level of infection varied among treatments for both studied cultivars. Compared to monocrop, intercropping with fenugreek reduced the total broomrapes per plant by 49.1% and 43.8% for cv. Badi and Najeh, respectively. These decreases were mainly expressed for the non emerged broomrapes (subterranean tubercles). In fact, decreases of 64.8% and 78% in subterranean tubercles were observed for cultivars Badi and Najeh, respectively when intercropped with fenugreek against reductions of 34.3% of emerged shoots observed for cv. Badi but no decrease was signaled for cv. Najeh.

## 92. Effect of Trap and Catch Crops on Egyptian Broomrape (*Phelipanche aegyptiaca*) in Tomato

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The aim of study was to determine the effect of some trap and catch crops to control Egyptian broomrape (*Phelipanche aegyptiaca* (Pers.) Pomel (syn. *O. aegyptiaca* Pers.)) in tomato. A pot experiment was carried out in 2008 in Adana, Turkey. The experiment was established with 6 replications in a climatic room with 23/25°C night/day temperature and 12/12 h dark/light settings. Cabbage, brussels cabbage, cauliflower, broccoli, turnip, canola, lentil and flax seeds were sown in pots. Approximately 2 months after sowing, plants were harvested, chopped into small parts and incorporated into the same soil. Tomato seedlings were planted in pots containing this mixed soil. Number of broomrapes branches, fresh and dry weights of broomrapes were evaluated. Broomrapes were counted periodically starting from flowering stages of broomrapes. After counting, broomrapes were cut at soil level, dried and weighed. During the experiment, counting and weighing broomrapes were done 9 times. At the end of the experiments total number of broomrapes emerged and their weight for each treatment were determined adding data from each individual count and weight porcesses. It was found that growing of turnip before tomatoes reduced dry weight of egyptian broomrape at the maximum percentage.

### 93. The management of dodder in central Iran

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Dodder (*Cuscuta campestris* L.) is a parasitic weed that decimates the foliage of several crops in central Iran. The legume crops such as camelthorn (*Alhagi camelorum*) are a susceptible host of the shoot holoparasite dodder in central Iran, and suffer severe yield losses due to high levels of infestations. A field study was conducted on 2007-2008 to evaluate the efficacy of glyphosate and glufosinate ammonium in controlling dodder in Shahed Medicinal Plants Research Park in Tehran Province, Iran. Results showed that glyphosate at 50-75 ppm controlled dodder, with minor phytotoxicity to the camelthorn. The application of glyphosate at 25 ppm controlled the dodder without apparent phytotoxicity to camelthorn. It was concluded that application of glufosinate at 50 ppm, sprayed on camelthorn showed control of dodder.

#### **94. Sunflower broomrape (*Orobanche cumana*) control in sunflower (*Heliantus annuus*) with glyphosate**

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Sunflower (*Heliantus annuus* L.) is a susceptible host of the root holoparasite sunflower broomrape (*Orobanche cumana* Wallr.) in the center of Iran, and suffers yield losses due to high levels of broomrape infestation. A field study was conducted in 2006 to evaluate the efficacy of glyphosate [N-(phosphonomethyl) glycine] for controlling broomrape in sunflower in Iran. In this study several rates and different times of application of glyphosate were tested. Nearly complete control of broomrape was achieved with glyphosate when it was sprayed twice when the broomrape attachments to the sunflower roots were at tubercle stage of development and 25 days later at a rate of application of 350 g a.i.ha<sup>-1</sup>. As an overall mean the herbicide was successful and increased the yield of sunflower by 65% and reduced number and dry weight of *Orobanche* shoots by 46.2 and 55.5%, respectively.

## 95. Leguminous crops as trap crops for *Striga hermonthica* control under field conditions

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42

The parasitic weed *Striga hermonthica* is the main biotic factor affecting sorghum, maize and millet production in the semi-arid tropics. Since there is no simple, fast and inexpensive measure for *Striga* control, intercropping with trap and catch crops seems to be the most accepted and environment friendly method for *Striga* control and of high benefit to the sustainable farmers. Two years field experiments were conducted under Sudan conditions to study the efficacy of *Dolichous Lablab* and varieties of *Vigna angiculata* on reducing *Striga* infestation. The leguminous crops were sown first then a month later followed by sorghum. In these experiments *Striga* emergence was found to be reduced by 98 – 100% compared to the control, resulting in increasing sorghum dry weight by 24-43% compared to the control, sorghum head weight up to 30%, in addition the legume crop yields ranged from 22-34 t/ha. *Vigna angiculata* was found to be the best in controlling *Striga* coupled with high yielding.

## **96. Generating parasitic plant resistant crops using a *Cuscuta* cysteine protease and a parasite inducible promoter**

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To prevent the infection of crops with plant parasites like *Cuscuta*, *Orobancha* and *Striga*, we investigated the interaction between these parasites and their host plants. We found a cysteine protease in *Cuscuta reflexa* and named it Cuscuin. Its expression is upregulated in *Cuscuta reflexa* haustoria during the attack on its host plant. The immature cysteine protease contains a propeptide which is cleaved after secretion of the enzyme. This activates the cuscuin. We could lower infection rates of *Cuscuta* by external application of the propeptide or by expression in host plants. Secondly we found a promoter of an arabinogalactan protein which is inducible by *Cuscuta* and *Orobancha*. Propeptide and promoter can provide useful tools for engineering resistant plants.

**HOST AND NON-HOST RESPONSES  
TO PARASITISM**

**97. Global gene expression profiling during resistant and susceptible interactions of cowpea with *Striga gesnerioides***

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Cowpea (*Vigna unguiculata* L. Walp.) is a major food and forage legume in sub-Saharan West and Central Africa. Among the major biotic constraints to cowpea production is parasitism by *Striga gesnerioides* (Willd.). At least seven distinct races of *Striga* parasitic on cowpea exist in West and Central Africa. Resistant cowpea genotypes exhibit two different response mechanisms to *Striga* attack, rapid necrosis at the site of parasite attachment (hypersensitive response - HR) and tubercle arrest (TA) in which there is very limited parasite growth and a failure to expand their cotyledons. The resistance response elicited in cowpea roots is dependent on the host genotype X parasite race interaction. We have carried out global gene expression profiling using a 385,000 feature cowpea microarray representing >45,000 gene coding sequences to examine the nature of gene expression in the interaction of the cowpea cultivar B301 with three different races of *S. gesnerioides* that elicit different responses: B301/SG3 - resistant/HR; B301/SG4z - susceptible; B301/SG6i - non-host resistance/TA. Gene expression profiles were compared at early and late stages of the resistant/susceptible interaction and both positive and negative changes in gene transcription were identified. A number of genes previously identified as being part of plant defense pathways were found to be highly up-regulated in the roots of cowpea undergoing a resistance response, including genes encoding pathogenesis related proteins, key enzymes in the lignin synthesis pathways, and enzymes of secondary metabolism. The results of this analysis and the implication of our findings to understanding the molecular genetic basis of cowpea resistance to *Striga* parasitism will be discussed.

## 98. The secret of broomrape host-specificity

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Host-specificity of *Orobanche* and *Phelipanche* spp. (the broomrapes) is differently expressed. There are species with only one or few hosts, others with a wide spectrum of host plants. It may be assumed that specific receptors exist in the broomrape seed, which are stimulated and start a cascade of reactions resulting in its germination. The assumption of specific receptors is supported by the strigolactone activities in minute concentrations, which require a receptor mechanism, and by the observation of specific lack of sensitivities, e.g. for GR24, a structural analogue to the strigolactones. The diversity of strigolactone structures provides a key for host specificity. Strigolactone receptors and host specificities are inherited. By hybridization the specificities from both parents are inherited, e.g. *P. lavandulacea* became a tobacco parasite by hybridization with *P. ramosa*. Is this also the case with *O. foetida* as a transient form between wild flora and agricultural pest? Hybridization is a rare happening in self pollinators, to which most *Orobanche* and *Phelipanche* spp. in the wild flora belong to. In a cross pollinator, like *O. crenata*, hybridization between genotypes is a rule. Since *O. crenata* shows many chromosomal aberrations, polymorphic hybrids with different host specificities are formed. The seeds of all of them form a mixed seed bank. For each legume cultivated on the infested soil, there are suitable *O. crenata* seeds fitting to that host! This mimics the broad host specificity. Recent analyses of the strigolactone composition of host plants from broomrape of the wild flora support the idea that for those species a specific bouquet of strigolactones is required for germination stimulation. When the specificity for the bouquet is lost, *Orobanche* and *Phelipanche* spp. may become agricultural pests. *O. cumana*, which only parasitizes sunflower, has probably evolved from *O. cernua*, which has a relative broad host spectrum. Their receptors must have lost the sensitivity for strigolactones, instead they respond to sesquiterpene lactones exuded by sunflower roots. Plants that exude strigolactones and stimulate the germination of weedy broomrapes but are not parasitized, need defence mechanisms. Polygalacturonase inhibitors or polygalacturonase-inhibiting proteins were good candidates.

## 99. Induction of phytoalexin biosynthesis in *Lotus japonicus* roots in response to *Striga hermonthica* attachment

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*Lotus japonicus*, a non-host of *Striga hermonthica* and a compatible host of *Phelipanche aegyptiaca* (syn. *Orobanche aegyptiaca*), was employed to study responses to early stages of parasitism. *L. japonicus* induced *Striga* germination and attachment, but did not support further development. On the other hand, *L. japonicus* successfully hosted *P. aegyptiaca* to complete development. cDNA libraries were established, enriched by suppression subtractive hybridization for transcripts up-regulated in *L. japonicus* root segments adjacent to the attachment points of *S. hermonthica* seedlings (Lj-Sh) and those adjacent to *P. aegyptiaca* tubercles. Genes involved in biosynthesis of vestitol, an isoflavan phytoalexin produced by *Lotus* and several other leguminous genera, were exclusively found in Lj-Sh. Expression of the genes related to vestitol biosynthesis was higher in roots supporting *S. hermonthica* attachment than in those parasitized by *P. aegyptiaca* and in the non-inoculated controls. Based on chromatographic behavior on HPLC and mass spectroscopic data, vestitol was identified in exudates released by *L. japonicus* roots inoculated with *S. hermonthica*. Fluorescence similar to that of authentic vestitol was observed on the surface of *L. japonicus* roots supporting *S. hermonthica* attachment, while such fluorescence was not detected on the non-inoculated roots. Vestitol inhibited radicle elongation in *S. hermonthica*. The results suggest that *L. japonicus* roots recognize *S. hermonthica* as an intruder and vestitol functions as a suppressant to prevent invasion. This work was supported, in part, by a grant from the AA Science Platform Program of the Japan Society for the Promotion of Science.

## 100. Multiple layers of nonhost incompatibility to *Striga hermonthica*

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*Striga hermonthica* infests crop plants and causes considerable yield losses in sub-Saharan Africa. It parasitizes gramineous plants including sorghum, millet, maize and upland rice, but not dicots. To understand its recognition mechanisms of hosts, we investigated its interaction with nonhost dicots including arabidopsis, cowpea, *Lotus japonicus* and *Phtheirospermum japonicum*, a hemiparasitic plant. *S. hermonthica* seeds were pretreated with strigol, a germination stimulant, allowed to germinate next to a potential host root, and co-incubated in a rhizotron chamber. All nonhost plants tested did not support *S. hermonthica* growth beyond the six leaf pair stage, but the arrest of parasite development occurred at various stages. *S. hermonthica* haustoria are able to reach the stele of *Arabidopsis* and cowpea, while *L. japonicus* blocks *S. hermonthica* infection in the root cortex. *S. hermonthica* often fails to penetrate *P. japonicum* roots. To investigate the interaction with *P. japonicum* in more detail, we monitored the infection of the host rice and the nonhost *P. japonicum* using time-lapse photography. Interestingly, *S. hermonthica* grew toward to the nonhost root, but turned away without making haustorium. This result indicates that *S. hermonthica* does not recognize *P. japonicum* root as a proper host in the stage before haustorium development. Our results suggest that there are multiple types of incompatible interaction to *S. hermonthica*. Combinations of these different incompatibility mechanisms contribute to the total resistance to *S. hermonthica*. In addition, we constructed a full-length-enriched *S. hermonthica* cDNA library and read the sequences from both ends of approximately 37,000 clones. The Expressed Sequence Tag (EST) sequences were clustered into non-redundant 17,000 unigenes and annotated using blastX program. Our EST data will be placed in a web site to be accessible worldwide.

**101. The response of two legume crops (hyacinth bean and kidney bean) to the parasitism of field dodder (*Cuscuta campestris*)**

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Microscopic examinations and chemical studies were performed to study the response of two legume crops, hyacinth bean (*Lablab purpureus* (L.) Sweet) and kidney bean (*Phaseolus vulgaris* L.) to field dodder (*Cuscuta campestris* Yuncker) parasitism. Hyacinth bean was found to be a highly susceptible host to *C. campestris* showing no effective resistance mechanisms. However, kidney bean was found to be an incompatible host displaying resistant reactions towards the parasitism of *C. campestris*. The possible reasons for the resistance of kidney bean to *C. campestris* were anatomical (hypersensitivity) and chemical (high contents of phenolic acids and lignin) stimulated defense mechanisms, which developed during the actual intrusion of haustorial cells inside its tissues.

## **102. Resistance mechanisms to *Orobanche crenata* in the model legume *Medicago truncatula*: The isoflavonoid response**

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Mediterranean area presents the ideal weather conditions for *Orobanche crenata* Forsk. (crenate broomrape) development, one of the major legume crop constraints. This obligate root holoparasite depends completely on its host for all nutritional requirements which it obtains by establishing host vessel connections through the developed of a special organ called the haustorium. The interaction between *O. crenata* and susceptible hosts have been well characterized but less is known about the basis of host resistance. In this sense, only some resistance mechanisms have been identified in certain legume crops such as faba bean, vetches and pea. Nevertheless, work with these crops is quite difficult because of its complex genome and physiology. Therefore, it is necessary to find models that permit easy work and identify genes expressed in this interaction or metabolic pathways activated against this parasite. For this reason, we have studied the response against *O. crenata* attack using *Medicago truncatula* to clarify the mechanism of resistance acting in this interaction. Previous reports have revealed that most legume crops present physical barriers as a defence against broomrapes but our results show that *M. truncatula* seems to defend itself mainly using chemical compounds i.e. isoflavonoids (phytoalexins). These phenolic compounds are well known in the plant kingdom thanks to their antifungal and antimicrobial activity. Here we describe some of the isoflavonoids acting against *O. crenata* invasion and quantify their concentration in host infested tissues using two *M. truncatula* accessions with different resistance reaction time. Thin-layer chromatography (TLC) plates revealed that both accessions set off medicarpin, maackiain and scopoletin apart of other isoflavonoids non identified yet; and staining root sections showed darkening and necrosis of host tissues and parasite intrusive cells at the infection points. These results suggest that *M. truncatula* produces and excretes these compounds as a toxic defence against *O. crenata* invasion.

**BREEDING FOR PARASITIC  
PLANT CONTROL**

### 103. Stability of *Orobanche* resistance of faba bean lines in various environments

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Faba bean (*Vicia faba* L.) is one of the most important pulse crops in the world with roughly 4.9 million tons annual grain production over 2.62 million hectares. Faba bean production is affected by various biotic and abiotic stresses. Broomrapes, i.e. *Orobanche crenata* cause considerable yield losses varying from 7 to 80 % in the main producing countries such as Ethiopia, Morocco, Tunisia, Egypt, Sudan, Syria, Turkey, Spain and Portugal. The broomrape infested area is increasing. In some areas, faba bean can also be infected by *O. foetida* or *Phelipanche aegyptiaca*. Host plant resistance is a major component in integrated broomrape management. Efforts were made at ICARDA in developing advanced breeding materials with acceptable tolerance to broomrape. Several lines with levels of resistance were selected under field conditions. This germplasm was sent to NARS to test and confirm the stability of broomrape resistance. Sixteen genotypes with acceptable levels of resistance were tested in various locations in Egypt, Sudan, Tunisia and Syria during the 2006 cropping season. Data on broomrape numbers and dry weight per square meter were obtained from the research collaborators. Multi-location analysis showed eight lines (F5/3423/03/Or4-7; F5/3791/03/Or4-3; F5/3043/03/Or4-14; F5/3382/03/Or4-4; F5/3621/03/Or4-8; F5/3791/03/Or4-3; F5/3087/03/Or4-14 and F5/3384/03/Or4-7) with a high resistance stability. The average yield of the chosen lines when exposed to broomrape infection was 15% lower than the average yield of uninfected susceptible checks. The introgression of those resistant lines into highly productive varieties will be an essential step to enhance productivity and resistance of faba bean.

#### **104. Evaluation of early and extra-early maize cultivars for their reaction to *Striga hermonthica* in the North-Western Nigeria**

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One of the successes of the maize revolution in Nigeria is the advent of extra early maize cultivars that are now being promoted in virtually all the drier regions of Nigeria. One of the major challenges to maize production in Nigeria is the problem of weeds, especially *Striga hermonthica*. It has been a continuous search not only for improved cultivars but for those cultivars that are resistant/tolerant to *Striga*. Consequently two early maize cultivars and two extra early cultivars were evaluated on station for their reactions to parasitic weed -*Striga hermonthica* at the University farm of Ahmadu Bello University, Zaria, Nigeria under natural *Striga* infestation between 1998 to 2004 followed by on farm evaluations in Kaduna and Zamfara states. The two early maturing cultivars had fewer number of emerged *Striga* but less vigorous, lower yield than TZE COMP-3 C 1 but were comparable to AB11. Grain yield was negatively correlated with *Striga* parameters. During the field evaluations improved Cultivars 99TZEEY and Syn. 2000 were introduced which were improvement on 95 TZEE Y and 95TZEEW respectively. In all the field observations, these new cultivars have shown high level of tolerance to *Striga* and appreciable yields ranging between 1.8-2.7 t/ha on the farmers fields. *Striga* is endemic in most of the drier regions where the extra-early maize is being promoted, therefore resistance to *Striga* is a vital factor for adoption.

## 105. Chemical mutagenesis and haploidy - combined approach for breeding broomrape resistant tobacco

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*Phelipanche ramosa* (L.) Pomel (syn. *Orobancha ramosa* L.) is the most widely distributed parasitic plant on tobacco in Bulgaria. There have been no naturally resistant *Nicotiana* species or tobacco cultivars found within tobacco germplasm till now. Here we present an approach for obtaining tobacco forms with increased resistance to broomrape by combining chemical mutagenesis and haploidy, which enable selection of mutagenic tobacco lines with all alterations (dominant and recessive), related to the host resistance to the parasite. Ethylmethanesulphonate (EMS) at concentrations 0.6 % was used for treatment of tobacco seed cv. Burley 21. The breeding process started with screening of haploid forms derived from anthers of M0 plants that remained noninfested in the test application for resistance to broomrape. After four consecutive tests conducted with the obtained haploids for resistance to broomrape, only one line was selected and dihaploidized in vitro by direct organogenesis from stem explants. The progeny from the seed of successfully obtained dihaploids was tested again for resistance to broomrape and ultimately four lines were selected that showed increased resistance. All screening tests were conducted in pots under greenhouse conditions and plants were grown in soil mixed with 200 mg seeds of *P. ramosa* per 1 L soil volume. Application of combined chemical mutagenesis and haploidy appears a promising approach for breeding of tobacco lines with increased resistance to broomrape

## 106. Screening of chickpea (*Cicer arietinum*) genotypes for Field Dodder (*Cuscuta campestris*) resistance

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Field dodder (*Cuscuta campestris* Yuncker) is a widely distributed shoot parasitic plant causing severe damage to various crops by attaching and penetrating their shoots and withdrawing assimilates, solutes and water. Attempts to manage this noxious weed using cultural, biological and chemical measures have yielded only limited success. Chickpea is the world's third most important food legume and is a major crop, food and source of protein grown on about 11 m ha, mainly in Asia and East Africa. Chickpea is highly susceptible to field dodder, however, available means to selectively manage this parasitic plant in the crop are insufficient. The aim of this study was to screen chickpea genotypes for variation in their response to field dodder in order to identify, understand and utilize tolerant/resistant genotypes. A wide collection (51 genotypes) of chickpea genotypes representing a wide array of regions around the globe, were tested for their association with field dodder. The experiments were held in pots in a controlled environment greenhouse in Rehovot, Israel. Each chickpea genotype was subjected to four treatments: control without dodder and seeding of dodder 0, 14 and 28 days after chickpea emergence (DAE). Each treatment was replicated three times. Chickpea development and dodder parasitism progress were carefully monitored and recorded twice a week. The experiment was terminated by separating dodder and chickpea plants and determining their fresh weights. Differences in field dodder parasitism on different chickpea genotypes were observed. Three genotypes showed good resistance to field dodder at all dodder inoculation periods. In four genotypes resistance was observed only at the third inoculation period when the plant matured and one genotype showed resistance only at the early inoculation date. The resistance phenomena observed was in most cases characterized by the failure of the pre-haustorium to penetrate the chickpea stem. Further studies are in progress to confirm the selected genotypes resistance and to determine the mechanism(s) involved in the resistance using histological and chemical techniques.

**107. Race-specific resistance of cowpea to *Striga gesnerioides* parasitism is conferred by a CC-NBS-LRR type R protein.**

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Cowpea (*Vigna unguiculata* L. Walp.) is a major food and forage legume in sub-Saharan West and Central Africa. Among the major biotic constraints to cowpea production is parasitism by the hemiparasitic angiosperm *Striga gesnerioides* (Willd.). Genotyping and host differential response studies have established that the parasite exhibits a high level of host specificity and that at least seven distinct races of *S. gesnerioides* parasitic on cowpea exist in West and Central Africa. A molecular marker (SSR-1) polymorphic in populations derived from crosses of resistant and susceptible cowpea genotypes, and tightly-linked to resistance to *S. gesnerioides* race 3 (SG3) was used to screen a BAC library from the SG3 resistant cultivar IT92D-499-35. A BAC clone (p20O21) was identified that contains a 10 kb (p20O21-E9) fragment encoding a R gene homolog containing the SSR-1 marker. This R gene, designated RSG3-301, encodes a R protein with an N-terminal coiled-coil (CC) domain, a nucleotide-binding site (NBS) and C-terminal leucine-rich repeat (LRR). Virus-induced gene silencing (VIGS) was used to knock down endogenous expression of RSG3-301 in SG3 resistant B301 cowpea plants. RSG3-301 knockdown plants do not develop a hypersensitive response and associated necrosis at the site of parasitism, symptomatic of the SG3 resistance response, but rather support growth of the parasite similar to susceptible plant genotypes. RSG3-301 mRNA is constitutively expressed in cowpea roots and overexpressed YFP tagged RSG3-301 protein appears to localize to the plasmamembrane. Our findings open the door for further exploration of the development and evolution of resistance in these unique plant-plant associations.

## 108. Regeneration and transformation method for faba bean

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Broomrapes (*Orobanche* spp.), the major uncontrolled parasitic weeds in the Mediterranean region, cause dramatic losses to many crops such as faba bean (*Vicia faba* L.). Genetic engineering may offer new insight and strategies to control these parasites. However, the utilization of genetic transformation techniques to introduce useful genes in faba bean requires an efficient regeneration and transformation method. In this context, an efficient and a rapid method of organogenesis in faba bean was developed with 100% of regeneration efficiency. This was based on the use of an adsorbent (activated charcoal) and antioxidants (polyvinylpyrrolidone [PVP], silver nitrate, ascorbic acid and cysteine) for reducing the browning of explants by phenolic compounds. We tested cotyledonary nodes, cotyledonary buds and epicotyls as explants for regeneration. Before culturing the explants on the media, we pre-treated the seeds with PVP, and germinated on half strength Murashige and Skoog media for six days. The study revealed that both adsorbent and antioxidants reduced the browning of explants and shoots were regenerated. However, higher percentage of regeneration was observed with media supplemented with activated charcoal and ascorbic acid and with cotyledonary node as explants. In general, ascorbic acid had given the highest number shoots/explant compared to activated charcoal. However, the regenerated shoots were longer and healthier in activated charcoal treatment. The cotyledonary node is the most suitable type of explant for regeneration of faba bean. *Agrobacterium*-mediated transformation of cotyledonary nodes of faba bean was established by using the pCGP 1258 plasmid. Fertile plants were regenerated from transformed explants and the integration of the Gus gene was confirmed by PCR. This protocol will be further used for *Agrobacterium*- mediated genetic transformation of a Sarcotoxin IA gene in order to enhance *Orobanche* resistance in faba bean.

## 109. Recent development of chemical control and breeding for broomrape resistance in sunflower

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Broomrape (*Orobanche cumana* Wallr.) has been one of the most important problems in sunflower in Turkey, Spain and some Eastern European countries for 50 years. Although new genetic resistance to this parasite was found in sunflower, new virulent *O. cumana* races occurred, overcoming these resistances. In addition to the five known races, named A through E and identified using a set of differentials carrying the dominant resistance genes *Or1* through *Or5*, respectively a new F race was found in the last 7-8 years and characterized by the *Or6* gene in sunflower. Additionally, some new races or sub-races have also been reported but not proven yet. Therefore, most research has focused on the development of new genetic resistance sources from wild types of sunflower utilizing classical and molecular genetic studies. On the other hand, great success was obtained by chemical control of broomrape in sunflower, after introducing resistance genes to the Imidazolinone (IMI) herbicides via backcross breeding methods. CLEARFIELD system, which is the combination of sunflower hybrids carrying resistance genes to broomrape and also to IMI herbicides, were applied for the first time in Turkey five years ago and increased market share rapidly both in Turkey and in other countries. In this system, IMI herbicide is applied post emergence to control all known races of broomrape and at the same time also to control some key weeds, such as *Xanthium strumarium* L., *Sinapis arvensis* L., *Chenopodium album* L., *Cirsium arvense* (L) Scop., *Convolvulus arvensis* L., *Avena* spp., *Datura stramonium* and *Amaranthus* spp. in sunflower fields. However, some hesitations have arisen regarding the potential danger of IMI residues in soil and also regarding the possible development of IMI resistance in both the weeds and the broomrape. Additionally, characterization studies of the genetic structure and variability of broomrape populations were performed to aid developing long-term strategies for broomrape management in sunflower.

## 110. Reactions of sorghum cultivars to *Striga hermonthica* in Zaria, Nigeria

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Eight sorghum cultivars were evaluated for their reactions to *Striga hermonthica*. The trial was conducted on the *Striga* infested fields of the Institute of Agricultural Research, Ahmadu Bello University, Zaria Nigeria. Cultivars of sorghum differed significantly in percent *Striga* infected plant, number of *Striga* counted and *Striga* severity symptoms at harvest, *Striga* count, crop vigour and grain yield. Cultivars ICSV 400, although supported more *Striga* emergence than ICSV 100BF, was the highest yielder in the first year of the trial and comparable to ICSV 1079 BF which was then highest grain yield in the second year. In the two years, ICSV 1007BF although exhibited high level of resistance, its yield was low. Grain yield was negatively correlated with *Striga*.

**111. Application of chemical mutagenesis to increase the resistance of tomato to *Phelipanche ramosa***

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The possibility of using chemical mutagenesis on tomato in order to increase the resistance to branched broomrape (*Phelipanche ramosa* (L.) Pomel (syn. *O. ramosa* L.) was studied. Seeds from Bulgarian tomato cultivar Bela were treated with ethyl methanesulfonate (EMS) and M1 plants were grown in the field. The response of a number 2467 M2 tomato lines to *P. ramosa* infection was evaluated in a large scale screening conducted in greenhouse. Tomato plants were grown in soil containing broomrape seeds and their roots were examined for the presence of developing parasites. As a result sixteen tomato plants non-infected by *P. ramosa* were selected. Their offspring were screened for resistance using the polyethylene bag system. The germination of *P. ramosa* seed was observed and comparison between the numbers of developed tubercles in the M3 progeny and the control plants was examined. Finally six lines showing significantly lower levels of infection were selected. The obtained results confirm that chemical mutagenesis gives a real possibility for increasing the resistance of tomato to broomrape. Since there is no natural resistance to the parasite in tomato or all *Solanaceae* species, this method could be a promising one to add such an important property in tomato cultivars.

**SPECIAL TOPICS 1:  
HEMIPARASITES**

## **112. Rhythms of nutational movement and seasonal changes in jasmonate levels during the course of the year and under constant conditions in mistletoe (*Viscum album*)**

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Some compounds present in mistletoe (*Viscum album* L.) show large seasonal variations correlating with distinct chronobiological phenomena. During the heterochronical cycle of bifurcated shoot development mistletoe plants show nutational movements with circadian periods and higher amplitude nutations. Both exogenous and endogenous factors seem to control these movements. In the final stage of the nutational movements occurring during summer time, the shoots change from a vertical position to a more radial one causing a typical spherical shape of the plant. During these changes high levels of the plant hormone jasmonic acid and its precursor 12-oxophytodienoic acid accumulate. In 2003 we determined a 100-fold increase in the levels during the nutational period compared to that four weeks before. Most of the jasmonic acid appeared as (+)-7-iso-jasmonic acid indicating its de novo synthesis. These results represent the first proof on the occurrence of jasmonates in mistletoe. Moreover, we registered an increased presence of AOC protein in vascular bundles, which is an essential enzyme in jasmonate synthesis. Both suggest a new role of these compounds in nutational movement. The seasonal changes in levels of jasmonate observed in 2003 were confirmed by analyses performed each year between 2004 and 2006. Under constant growth conditions in climate chambers, high levels of both jasmonic acid and 12-oxophytodienoic acid were found. Moreover, altered levels of jasmonate occurred during the course of the day. The flexibility of mistletoe in terms of jasmonate levels is also supported by a wound-induced accumulation of jasmonate, a well-known phenomenon for most plants. Earlier studies have showed that jasmonic acid induces apoptosis and suppresses cell proliferation in more than 15 human cancer cell lines leading to a public acceptance on the anti-cancer effect of jasmonates. Consequently, our data support a link between anti-tumor activity of mistletoe and anti-cancer activity of applied jasmonates.

### 113. Effect of host interaction on the phytochemical composition of *Helicanthus elastica*

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*Helicanthus elastica* is the most virulent hemiparasite attacking most of the tree species of the tropics. In Ayurvedic medicine the medicinal properties of the mistletoes are ascribed to their host plant. Ancient literature states medicinal properties for loranthaceae members collected from specific trees. A study was undertaken to screen the phytochemical composition of *H. elastica* collected from thirteen different host plants. 25 g of the powdered material was serially extracted with 125 ml each of petroleum ether, chloroform, ethyl acetate, ethanol and water in the increasing order of polarity. Qualitative tests were conducted to detect the presence of secondary metabolites in the extract. The chemical composition of the host plants showed a lot of similarity however some differences were also noticed. The petroleum ether extract tested positive for tannin and phenols only in the case of *Manilkara zapota*, *Chrysophyllum cainito* and *Spondias indica*. Flavonoids were present in *Syzygium cumini*, *Callistemon citrinus*, *Hibiscus rosa-sinensis* and *Mangifera indica*. Steroids were seen in *Theobroma cocoa* and *Anacardium occidentale* while cardioglycosides were present in *Hevea brasiliensis*, *Pimenta dioica*, *Anacardium occidentale* and *Theobroma cocoa*. Terpenoids were absent in *H. brasiliensis*, *P. dioica*, *Myristica fragrance* and *M. indica*. Chloroform extract also behaved in a similar manner saponins, tannins and flavanoids were detected only in the case of *S. cumini*, *C. citrinus* and *M. indica*. Flavanoids and terpenoids were detected in the case of *H. rosa-sinensis* and *S. indica* all others had very little secondary metabolites. In ethyl acetate extract saponins, tannins and phenols flavanoids and terpinoids were the only secondary metabolites detected. However in rubber tannins and phenols was not detected. Maximum phytochemical were detected in ethanol and water extracts. Flavanoids from the ethanolic extract was detected only in the case of *S. cumini* and *H. rosa-sinensis*. Alkaloid were present in *H. rosa-sinensis*, *S. cumini*, *P. dioica*, *Alianthus sp*, *M. fragrance*, and *A. occidentale*. Terpenoids was absent in the ethanol extract of *T. cocoa*. Other secondary metabolites like cardioglycosides, saponins, tannins and phenols were present in all the species tested. Water extract also tested positively for saponins, alkaloids, tannins and phenols, terpenoids and cardioglycosides. However tannins and phenols were absent in the water extract of *H. brasiliensis* and *T. cocoa*. Cardioglycosides was absent in the extract of *A. occidentale*. These finding confirm the popular belief that the medicinal properties of mistletoes are host specific.

## 114. Linking parasitic plant-induced host morphology to tritrophic interactions

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We investigated the tritrophic interactions among southwestern dwarf mistletoe [*Arceuthobium vaginatum* (Willd.) Presl subsp. *cryptopodum*], mistletoe herbivores, and host pine (*Pinus ponderosa* Dougl. ex Laws. and C. Laws. variety *scopulorum* Engelm.)-associated predators. In an observational study, we characterized differences in pine-associated arthropods and pine branch morphology between branches either parasitized by mistletoe (brooms) or not visibly infected. Compared with noninfected branches, brooms had a more reticulate branching structure, collected 36 times more dead needles and supported 1.7 times more arthropod predators. In a manipulative field experiment, we investigated whether pine-associated predators fed upon lepidopteran herbivores of mistletoe and thereby reduced herbivore damage to the parasite. Over a 30-day-trial, herbivores fed upon approximately two-thirds of available mistletoe shoots. Predator removal increased herbivore survival by 56% but had no detectable effect on the level of herbivory damage. We speculate that herbivores compete for mistletoe shoots and that increased per-capita feeding compensated for predator reduction of herbivore abundance. In summary, our results demonstrate that mistletoe parasitism altered the pine arthropod community, including an increase in the density of predators that likely feed upon mistletoe herbivores.

## 115. Modifications in wood anatomy caused by the mistletoe *Struthanthus vulgaris* in the host *Tipuana tipu* in Sao Paulo, Brazil

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Mistletoes usually have higher transpiration rates than their hosts and they are entirely dependent on them to obtain water. This situation may cause competition for water and water stress, which can affect host fitness. Our goal was to investigate whether the wood anatomy of *T. tipu* has been modified by the infestation of *Struthanthus vulgaris*. Parasitized and non-parasitized branch segments were sampled in the campus of University of Sao Paulo in Sao Paulo city, Brazil. *T. tipu* has a semi-ring-porous wood. This type of secondary xylem has a vessel size gradient between the early and latewood. In the rain season, the plant has high water availability and produces earlywood with wider vessels. When the dry season comes the vessels diameter decreases, therefore the latewood has smaller vessels than the early wood. Parasite plants can cause more effects on their hosts when the latter is more susceptible and this can occur when the plant has limited water availability. Therefore, we investigated if vessel elements area, frequency and diameter of parasitized and non-parasitized branch segments were modified by the mistletoe. Parasitism affects more vessel area and vessel frequency in the latewood than in earlywood. Vessel area can be three times smaller in the latewood of parasitized segments than in non-parasitized. The vessel frequency in non-parasitized segments is not different between earlywood and latewood, but for infested trees there are twice less vessels in latewood than in earlywood. Parasitized branches have smaller vessel diameters both in earlywood and latewood, but vessel area and vessel frequency were proportionally more reduced in the latewood than in the earlywood. We conclude that *S. vulgaris* modified the wood anatomy of *T. tipu* more intensively at the latewood. It seems that during the dry season the effect of mistletoe drainage is felt more intensively by the host than during wet season. Probably this causes a stronger reduction of the cell turgor at the vascular cambium than in wet season, because the water availability is lower. The modifications caused by the mistletoe may decrease the xylem hydraulic conductivity of *T. tipu* branches. These anatomical modifications were more significant when the plant has less water availability, which might be responsible for the death of some branches, which was observed in many trees. Beyond that we observed that parasitized branches have narrower annual rings.

## 116. Impact of hemiparasitic species *Rhinanthus minor* on grassland diversity

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*Rhinanthus minor* L. is found in a wide range of grassland habitats in the Serbia, being typically associated with hay meadow communities with a high floristic diversity. Influence of hemiparasitic species *R. minor*, grown in meadow communities *Danthonietum calycinae* Cinc. et Kojic and *Agrostietum vulgaris* Pavl. on Mt. Stol (Eastern Serbia), on productivity and species richness was investigated. The aim of this study was to determine its host range and selectivity and to evaluate its significance and contribution as a valuable resource, as it opens up the sward of grass, allowing other plants light and nutrients to grow. The two investigated mesophyllous meadow communities were dominated by grasses *Arrhenatherum elatius*, *Holcus lanatus*, *Festuca pratensis*, *Dactylis glomerata*, *Danthonia calycina*, and *Agrostis vulgaris* and leguminosae *Trifolium montanum*, *T. Alpestre*, and *Lathyrus pratensis*. The presence of *R. minor* in grassland is strongly related to soil fertility (productivity). *R. minor* was found to be more frequent in *A. vulgaris* than in *D. calycinae*. It was frequently associated with the following species: *F. pratensis* (80%), *P. pratense* (59%), *Alopecurus pratensis* (55%) and *Ranunculus acris* (58%). Only three species were found to be significantly positively associated with *R. minor*: *Plantago lanceolata*, *Festuca rubra* and *L. pratensis*. In contrast, 20 species were significantly negatively associated with *R. minor*. By examining the number of haustorial attachments between host and parasite (based on the relative haustorial frequency to host root weight), it was suggested that *R. minor* showed host preference in the following order *Trifolium*, *Festuca*, and *Holcus*.

## 117. Hemiparasitic plants of the humid tropics of India

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Eleven species of hemiparasitic plants belonging to the family Loranthaceae and six species from Viscaceae were collected from the humid tropical region of Kerala, India. The Loranthaceae members belonging to the genus *Helicanthus*, *Helixanthera*, *Scurrula*, *Taxillus*, *Macrosolen* and *Dendrophthoe* were found infecting the fruit trees and plantation crops of the state. Among the Viscaceae members, all from genus *Viscum*, *V. capitellatum*, *V. articulatum*, *V. angulatum*, *V. orientale*, *V. monoicum* and *V. ramosissimum* were observed. Among these only *V. orientale* was common in the plains. The others were seen in the high mountain ranges. Many of the *Viscum* spp. were found on forests while *V. orientale* and *V. capitellatum* were seen on road sides. Among the Loranthaceae members *Helicanthus elastica* was the most virulent and fast spreading species of the region infecting most of the fruit crops, which *Mangifera indica* was the most effected trees. However, this species was not seen at an altitude above 650 m. *D. falcata* was the most widespread species with the broader host range. It was seen both in the high ranges and plains. Both *Macrosolen capitellatum* and *M. parasiticus* were found in the plains and high ranges but while *M. capitellatum* was mainly detected on *Artocarpus heterophyllus*, *M. parasiticus* was common on *Terminalia* sp. *Taxillus cuneatus* was common in the high ranges but it was also rarely seen in some locations in the plains. *Scurrula parasitica*, *Taxillus tomentosus*, *D. neelgherrensis*, *D. trigona*, *D. memecylifolius*, and *Helixanthera intermedia* were the major species of the high ranges (600 m above sea level).

### **118. Identification and distribution of mistletoe and possible biological control agents in Sierra de Arteaga, Coahuila, México**

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Sierra de Arteaga is a forest at State of Coahuila, Mexico composed by very important ecologic species. It represents a source of economic incomes for the locals and also represents a touristic and recreational place. Mistletoes from the Loranthaceae and Viscaceae families are the most important parasites of the conifers in Canada, United States and Mexico. Species from the genus *Psittacanthus*, *Phoradendron*, and *Arceuthobium* causes the largest economic and ecological impact in the country. Mistletoe represents a serious danger for the survival of Coahuila's forests, regrettably there is not enough information available for the management of these parasitic plants. The objectives of this research were the identification and distribution of mistletoe species and the identification and evaluation of biological control agents. Samples were taken from three different regions in the Sierra de Arteaga: Los Lirios, Jame and San Antonio de las Alanzas in Arteaga, Coahuila, Mexico. Mistletoe collection was continued until two *Phoradendron* species were found attacking *Quercus*, *Pinus*, *Juniperus*, and *Abies*. A fungus was found causing anthracnose in both mistletoe species, this fungus was isolated and purified by monosporic culture. At microscope view dark brown, subglobose, thin-walled fungus fruiting body (ascocarp) containing asci and ascospores were found indicating the typical *Glomerella* (Teleomorph: *Colletotrichum*) perithecium characteristics; hyaline unicellular ascospores.

### 119. Specificity and preference of the mistletoe *Struthanthus vulgaris* (Loranthaceae) for urban tree hosts in Sao Paulo, Brazil.

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Some mistletoes are capable to infest many host species from different families. Loranthaceae, the biggest family of mistletoes has a tropical and subtropical distribution, and some genera with this capacity as *Struthanthus*. *Struthanthus vulgaris* is one of the broadest distributions in South and Southeast of Brazil. 700 trees were analyzed in relation to the following aspects: tree species, presence or absence of the hemiparasite and when present, the number of individuals per host. Only 8 tree species out of 57 growing in the campus were infested by *S. vulgaris*. This represents 12.5 % of the total species number. *S. vulgaris* infests 13% of the campus's trees. The infested species are: *Jacaranda mimosifolia* (Bignoniaceae), *Piptadenia gonoacantha* (Leguminosae), *Syzygium jambos* (Myrtaceae), *Syzygium cumini* (Myrtaceae), *Morus nigra* (Moraceae), *Caesalpinia pluviosa* (Leguminosae), *Psidium guajava* (Myrtaceae), and *Tipuana tipu* (Leguminosae). The last one has the highest infection rate by the mistletoe (30%). Furthermore, 54.66 % of the infested *T. tipu* exhibit more than one parasite growing in its branches, which represents 16.4 % of the entire population of the *T. tipu*. More specifically, from the 198 individuals of *S. vulgaris* found in the campus, 168 of them were growing on *T. tipu*, which represents 84,84% of mistletoe's population. We conclude that *S. vulgaris* is capable to infest different plant species and it seems to be a generalist parasite. However, if we want to understand the occurrence of the mistletoe it is necessary to consider some host characteristics that facilitates its installation, such as bark rugosity and thickness, the relative abundance of the host, and the behavior of the dispersive agents (in this case, birds). Assuming that, except for *Caesalpinia pluviosa*, all of the hosts plants are exotic, we may assume that they did not coevolved with the mistletoes, so they might not have any mechanism of defense against the parasite, making its infestation easier.

**SPECIAL TOPICS 2:**  
***Orobanche cumana***

## 120. Implication of HaDEF1 defensin in sunflower resistance to *Orobancha cumana*

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*Orobancha cumana* Wallr. is a major constraint for sunflower production in the Mediterranean regions. Development of resistant hosts is a promising way to control this parasitic plant but a better understanding of resistance mechanisms towards broomrapes is still necessary. For this purpose we have previously isolated a sunflower line, LR1, resistant to *O. cumana*. One component of its resistance results in necrosis of the attached parasites. We obtained data suggesting a major role of a defensin, HaDEF1, in this resistance. Indeed, the expression of the defensin gene, HaDef1, was strongly up-regulated during the response of the LR1 genotype to this parasite. Defensins are small basic peptides of about 50 amino acids mainly known for their antifungal activity. A recombinant peptide was therefore produced to study HaDEF1 activity on broomrape. While HaDEF1 toxicity towards fungi was found to be low compared to other plant defensins activity, it acted at much lower concentration (nanomolar) on broomrape seedlings by inducing cell death at the radicle apex. Under the same conditions, no lethal activity of HaDEF1 was detected on *Striga hermonthica* or on *Arabidopsis thaliana* seedlings. We have also demonstrated that HaDEF1 and the AAL toxin, a sphingolipid metabolism inhibitor, caused similar necrotic symptoms on *Orobancha* seedlings. Moreover, HaDEF1 necrotic activity on *O. cumana* was strongly inhibited by calcium addition in the medium. The use of the fluorescent calcium probe, Indo1-AM, revealed that HaDEF1 peptide triggered a rapid increase in intracellular calcium levels in *Orobancha* cells that could explain cell death. Pharmacological studies indicated an implication of IP3-regulated intracellular calcium channel in this response. The hormonal regulation of HaDef1 gene expression in sunflower was also studied. Interestingly, the HaDef1 gene was shown to be up-regulated by ABA. The implication of this drought-related hormone thus suggests that abiotic signalling pathways could be triggered in resistant sunflower in response to broomrape, as it has been demonstrated in other plant-parasitic plant interactions.

## 121. The mutation breeding for broomrape resistance in sunflower

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*Orobanche cumana* is a parasite of sunflower greatly affecting seed production. Huge efforts have been invested to find new sources of resistance utilizing wild types, interspecific hybrids or by chemical and radiation mutagenesis. The research described by us was conducted to develop resistance against new virulent races utilizing mutagenesis. These races cover over 80% of Trakya Region (with 75% of the sunflower production in Turkey). In this study, 150 and 200 gray doses from Cobalt 60 sources were applied to generate variation in sunflower inbred lines developed by National Sunflower Project conducted in Trakya Agricultural Research Institute, Edirne, Turkey. Based on the results, 200 gray dose was found the most efficient. 1000 seeds from female line 2453-A, restorer line 01001-R and open pollinated variety Vinimik 8931 were treated with these doses in 2002. The treated seeds were planted in soil and plants without deformations were selected. The progeny from from these plants were planted at three locations (Karabulut, Edirne; Malkara, Tekirdag; and Sutluce, Kirklareli) in naturally infested fields in Trakya region during 2003 and resistant plants were selected. Their seeds were bulked and planted in Karabulut and Malkara locations in 2004 and self pollinated. The same process for each line was repeated until 2008 in natural conditions in summer and artificial conditions in the pots during winter season. However, in other lines, 2453-A (female line) and Vinimik 8931 populations, the new resistant inbred lines were developed utilizing backcross and inbreeding methods. The seeds of these resistant lines will be multiplied and new resistant hybrids will be developed by crossing these lines in 2009.

## 122. Resistance and sensitivity in the parasitic system *Helianthus annuus* - *Orobanche cumana*

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Sunflower broomrape (*Orobanche cumana* Wallr.) is one of the most important constraints on sunflower production in Eastern and Southern Europe, Spain and parts of Asia. Yield losses resulting from a severe attack can reach 50-90%. The rapid evolution of broomrape populations, which leads to the occurrence of new virulent races, requires a continuous search for new sources for resistance. The study of *Orobanche* population genetics is of great importance for the understanding of the variability within and between pathogenic populations, helping in the selection programmes for developing sources of resistance. Ten populations of broomrape (*O. cumana*) have been analysed using RAPD markers. The seeds harvested from various countries were cultivated with a susceptible sunflower inbred line. The results allowed division of the populations in three groups. The genetic distance was not correlated with the geographic distance. The same ten broomrape populations have also been studied with the sunflower differentials (lines or hybrids) for the different races of the parasite. We found that the new more virulent populations, which developed in the last 3-4 years, are not significantly different from each other. Results of evaluation of sunflower germplasm for resistance to the different broomrape populations have demonstrated that the cultivated sunflower germplasm could still to be a valuable source of resistance genes. Most resistant sources have been found to be controlled by major genes, different dominance reactions, depending on the broomrape population and the source of resistance. Race-specific dominant genes are considered as good sources of resistance, but in recent years we have combined vertical and horizontal resistance, in the same sunflower hybrid, and also combined the genetic resistance to broomrape with genetic resistance to IMI herbicides.

### 123. Genetic diversity of *Orobanche cumana* populations from Spain and Eastern Europe

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Sunflower is one of the most important annual oilseed crops in the world. *Orobanche cumana* Wallr. (sunflower broomrape), a holoparasitic angiosperm plant that infects sunflower roots, is regarded as one of the main constraints on sunflower production in Spain and most countries of Southern and Eastern Europe and the Middle East. In this area, there has been a progressive development of this parasitic plant in sunflower crops and a rapid appearance of new and more virulent races which are not controlled in many cases by the resistance genes. Consequently, the survey and understanding of the evolution of broomrape populations and their genetic variability is essential for the establishment of efficient breeding programs. The aim of the present study was to characterize the genetic variability among 20 *O. cumana* populations from Spain and Eastern Europe using RAPD markers. *O. cumana* populations infected in their origin sunflower crops from different regions of Europe: Spain (Andalucía-Southern Spain and Cuenca-Central Spain) (10 populations), Bulgaria (6 populations), Romania (1 population), Serbia (1 population) and Turkey (2 populations). Thirty-five primers were used to obtain 98 polymorphic and reproducible bands which led to a binary matrix. The size of the scored amplified fragments ranged from 450 to 2000 bp. The UPGMA cluster analysis based on Dice index matrix showed a clear differentiation among *O. cumana* populations according to their geographic origin. Populations were well structured and organized into four distinct groups, two groups corresponding to the Eastern European countries (one group more homogenous formed by 4 Bulgarian and 1 Turkish populations, and other group more heterogeneous formed by Bulgarian, Turkish, Rumanian and Serbian populations), other group related to the previous ones and conformed by the Spanish populations of Andalucía, and finally the populations of Cuenca (Central Spain) in a last group more distanced of the others previously mentioned. Populations-specific markers were identified and several bands were only present in particular populations. Possible explanations for the distribution found are discussed.

## 124. Spreading and virulence of *Orobanche cumana* on sunflower in Rostov Region of The Russian Federation

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Sunflower is the main highly profitable oil crop and the Rostov region is an area of its intensive cultivation in the Russian Federation. Here, sunflower is often cultivated in the same fields for up to three consecutive years, at least during last 15 years. 43 districts of this region were inspected in 2008, and only in seven zones broomrape (*Orobanche cumana* Wallr.) was not found on sunflower. In all other districts broomrape was observed at various severities. In nine districts there were fields with 1-10 broomrape stems per square meter. In five districts the fields were infested with more than ten stems per square meter. In recent years the broomrape populations attacked sunflower varieties and hybrids which were resistant to *O. cumana* races D and E. The analysis of broomrape virulence in 12 districts was done using the following sunflower differentials: 7A and 202A (for race C), LC1002 (for race D), LC 1003 (for race E), LC 1093 (for race F), 16Ax25 (for race G) and P 96 (for race F). Sunflower plants were grown in climatic chambers, in boxes with mixture of soil and sand. 200 mg broomrape seeds were applied per 1 kg of soil mixture. 35 days after seedlings emergence, the amount of healthy broomrape tubercles was counted on the roots. All differentials were infected. Broomrape from districts Belokalitvinskyi, Egorlykskyi, Konstanti-novskiyi and Tastinskyi strongly infected 16Ax25 at 31-35 tubercles per affected plant. Broomrape from districts Kuibyshevskiyi, Millerovskiyi, Morozovskiyi, Rodionovonesvetaevskiyi and Stimlyanskyyi infected this line at a rate of 3-8 tubercles per plant. This line was resistant only to broomrape samples from Salskiy and Stelinskyi. A broomrape population from Turkey, containing races F, G, H, infected this line at 4 tubercles per a plant. Thus we conclude that a broomrape biotype, which is more virulent than race G, is widely distributed in some districts of the Rostov region.

## **125. Selection of sunflower (*Helianthus annuus*) inbred lines resistant to broomrape**

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Orobanche species are problem in many crop species in Iran and Turkey. *Orobancha cumana* is mostly seen on sunflower. There is not a clear evidence that when *O. cumana* was entered to Turkey. It is estimated that this species was introduced 80 years ago. The research was conducted on the experimental field of the Department of Field Crops, Faculty of Agriculture, and University of Ankara. The experiment was arranged randomized block design with four replications. The aim of the research was to determinate of inbred lines of V.8931 and Ekiz1, and find out their various characters such as resistance to broomrape, hull percentage, and oil percentage in 5<sup>th</sup> generation selfed sunflower lines. In inbred lines of Ekiz1/1, Peredovik/1, and Peredovik/3, broomrape was not found. Frequency was found between 1-10 % in inbred lines of V.8931/1, V8931/2, V8931/3, V8931/4, Ekiz1/3, V1646/2, V1646/3, V1646/4, and Peredovik.

## AUTHOR INDEX

### A

ABBASHER A.A. , 64, 113  
ABBES Z. , 31  
ABDELWAHD R. , 128  
ABRAHAM C.T. , 134, 138  
ABU ELGASIUM M. , 65  
ACHDARI G. , 79  
ACIC S. , 59, 137  
Ahmed E.A. , 113  
AKINNOLA N.A. , 123  
AKSOY A. , 82  
AKSOY E. , 82, 88, 110  
ALBERT M. , 114  
ALIAS Z. , 40  
AL-KHATIB K. , 96  
ALMANSOORI T.A. , 30  
ALTOMARE C. , 98  
AMRI M. , 63, 109  
ANNUAR M.S. , 40  
ANTONOVA T. , 146  
ARASLANOVA N. , 146  
ARDELEAN A. , 47, 117  
ARMAĞAN G. , 82  
ARSLAN M. , 82  
ASAM T. , 75 , 76  
ASAMI T. , 75 , 76  
AWANG K. , 40  
Azary A. , 135

### B

BABIKER A.G.T. , 65  
BAEVA G. , 102  
BAKAR B.B. , 40  
BAMBA T. , 27  
BASTIAANS L. , 96  
BAŞARAN S. , 82  
BATCHVAROVA R. , 86,125,131  
BAYE Y. , 54 , 106  
BAYRAKTAR O. , 82  
BENHOUHOU S. , 52  
BIJOY P.O. , 138  
BLEISCHWITZ M. , 114  
BOLIN J.F. , 19, 43

BOUHACHE M. , 33,34,54,108  
BOUWMEESTER H. , 67, 68, 89  
BOUYA D. , 33, 34, 108  
BOZ O. , 82, 88  
BOZDOĞAN O. , 82, 88  
BOZIC D. , 73  
BOZUKOV H. , 102  
**BRASIL B. de A.**, 44  
BRYL R. , 46  
BUFLASA A. , 30  
BÜKÜN B. , 82  
BÜLBÜL Z.F. , 82, 97  
BÜYÜKKARAKUŞ L. , 82

### C

CADISCH G. , 84, 85  
CARDOSO C. , 68  
CASTILLEJO M.A. , 21  
CECCANTINI G. 32,44,136,140  
CEPEDA PUENTE M.G. , 139  
CHABI W. , 31  
CHARNIKHOVA T. , 68  
CHEN G. , 35  
CHIKOYE D. , 94  
CHINNUSAMY C. , 48, 51, 93  
CIMMINO A. , 74  
CIUCA M. , 144  
CÍPRIANI M.G. , 98

### D

DAS M. , 18  
DE B. , 91  
DEHAGHI M.A. , 87, 103, 104  
DELAVAUULT P. , 26, 142  
DEMBELE S. , 96  
DEMIR A. , 82  
DEMİRKAN H. 82 , 90, 101  
DENEV I.D. , 23, 24  
dePAMPHILIS C. , 18  
deZÉLICOURT A. , 142  
do AMARAL M.M. , 32  
DOĞAN N. , 82  
DOMEIGNOZ L. , 136, 140  
DORKA R. , 133

DRAIE R. , 26

## E

ECONOMOU G. , 39, 55  
EIZENBERG H. , 79  
Elagab M.A. , 64  
ELZEIN A. , 84, 85  
ER T. , 105  
ERBAŞ F. , 82  
ERİLMEZ S. , 99  
EVCI G. , 129, 143  
EVIDENTE A. , 74  
EYMIRLI S. , 82

## F

FARAH A.F. , 120  
FEN B. , 84  
FERNÁNDEZ-APARICIO M., 21, 74  
FERNÁNDEZ-MARTÍNEZ J.M., 145  
FILIZADEH Y. , 111 , 112  
FUJII Y. , 40  
FUKUSAKI E. , 27

## G

GABOUN F. , 22  
GAL-ON A. , 28  
GBEHOUNOU G. , 38  
GEORGIEVA K. , 77  
GHOTBI Ma. , 87, 103, 104  
GHOTBI Mi. , 87, 103, 104  
GIRIJA T. , 134, 138  
GOLDWASSER Y. , 126  
GOWDA B. , 18  
GUCER T. , 143  
GUCHETL S. , 146  
GUI-LIN C. , 29, 72  
GUNATHILAKE P. , 17 , 18

## H

HADDAD A. , 92  
HADI H. , 103, 104

HAKAM N. , 128  
HAMAMOUCHE N. , 128  
HANADA A. , 75, 76  
HARRIS S.A. , 20  
HASHIM H. , 40  
HAUSE B. , 133  
HELLER A. , 85  
HermY M. , 46  
HERSHENHORN J. , 79  
HISCOCK S.J. , 20  
HOENIGES A. , 47, 117  
HONAAS L. , 18  
HRISTEVA T. , 77  
HRISTOVA E. , 45  
HUANG K. , 18, 116

## I

ISIK D. , 58, 60  
ITO S. , 75

## J

JAMIL M. , 68  
JOEL D. , 5, 15, 69  
JOSEPH B. , 27

## K

KACAN K. , 82, 90, 99, 101  
KADIOĞLU İ. , 82  
KALDENHOFF R. , 114  
KALYVAS D. , 39  
KANAMPIU F. , 70, 80  
KAPRAN I. , 96  
KAROĞLU S. , 82  
KATO A. , 75, 76  
KAYA E. , 58, 60, 82  
KAYA Y. , 129, 143  
KAYEKE J. , 49  
KAYENTAO M. , 96  
KHARRAT M. , 31, 63, 109  
KIEPE P. , 38  
KIRUBI D. , 80  
KITAHATA N. , 75  
KOBAYASHI A. , 27  
KOHLEN W. , 68

KOHLSCHEID E. , 71, 86  
KOLÖREN O. , 82  
KONSTANTINOVIC B. , 100  
KOSTOV A. , 61  
KOSTOV K. , 131  
KOTOULA- SYKA E. , 55  
KROSCHER J. , 84  
KYOZUKA J. , 75

## L

LACHNIT M. , 114  
LANDE T. , 79  
LAPIDOT M. , 28  
LEIBMAN D. , 28  
LI J. , 127  
LIS K.E. ,18, 116  
LOZANO-BAENA M.D. , 121  
LYRA D. , 39, 55

## M

MAALOUF F. , 123  
MAASS E. , 19, 43  
MACUKANOVIC-JOCIC M. , 59,  
137  
MAINSON D. , 142  
MALLORY-SMITH C. , 52  
MARLEY P. , 84  
MEHRVARZ S.S. , 41  
MELAN K. , 82  
MENNAN H. , 82  
MENTAG R. , 22  
MESELDZIJA M. , 100  
MEULEBROUCK K. , 46  
MIERSCH O. , 133  
MLAYEH O. , 63  
MOGHADDAM A.R. , 87, 103  
MORETTI A. , 98  
MOTLEY T.J. , 19  
MOUNA S. , 92  
MUBARAK N.E. , 65  
MUCHIRI N. , 80  
MUELLER-STOEVER D. , 71, 86  
MUKHTAR I. , 36  
MUMINJANOV H. , 62  
MUSSELMAN L.J. , 19, 30

## N

NAGLIS A. , 28  
NARASIMHA RAO RAO P. , 50  
NAUMOVA S. , 77  
NDAMBI B. , 85  
NEMLI Y. , 57, 82, 105  
NOMURA T. , 70

## O

OCHANDA N. , 96  
OGÜT D. , 82  
OKAZAWA A. , 27  
OMANYA G. , 80  
OTAMBEKOVA M. , 62  
OTHMAN M.R. , 40  
OUALLAH M.Y. , 52  
OZASLAN C. , 82  
OZDEMİR S. , 99  
OZTEMİZ S. , 82

## P

PACUREANU-JOITA M. , 144  
PALA F. , 82  
PEKCAN V. , 143  
PÉREZ-DE-LUQUE A. , 121  
PÉREZ-VÍCH B. , 145  
PÉRON T. , 26  
PETT B. , 62  
Pineda-Martos R. , 145  
PLAKHINE D. , 69  
POUVREAU J.B. , 26, 142  
PRABHAKARAN N.K. , 48, 51, 93  
PRATS E. , 121  
PRESS M. , 81  
PROCOPOVICI E. , 144

## Q

QASEM J. , 42  
QATTOF I. , 92

**R**

RAHMANI F. , 33  
RAICEVIC V. , 73  
RAMA RAO P.V. , 50  
RAMAZANOVA S. , 146  
RATHIKA S. , 48, 51, 93  
REHKER J. , 114  
RICHES C. , 49  
RODENBURG J. , 38, 49  
RONNEAU C. , 34  
RUBIALES D. , 21, 74, 121  
RUBIN B. , 126  
RUMSEY F.J. , 20  
RUŞEN M. , 53, 82  
RUYTER-SPIRA C. , 68

**S**

SADIDI F. , 112  
SAFFOUR K. , 34, 108  
SALAMI I. , 96  
SÁNCHEZ ARIZPE A. , 139  
SARIC M. , 73  
SATOVIC Z. , 21  
SAUERBORN J. , 71, 86  
SAVA E. , 144  
SCHOLES J. , 81  
SEID K. , 123  
SELLAMI, 109 F.  
SELOSSE M.A. , 14  
SERAJ AA. , 135  
SEREME P. , 95  
SEYMOUR R.S. , 43  
SHAABAN K. , 123  
SHAAR M.A. , 92  
SHANG-WU J. , 29, 72  
SHEAFFER L. , 17, 18  
SHINDROVA P. , 61  
SHIRASU K. , 119  
SIBONY M. , 126  
SIEVERDING E. , 71  
SIMIER P. , 26, 31, 142  
SINHA A.C. , 91  
SITALO G. , 146

SLAVOV S. , 125, 131  
SMIRNOV Y. , 79  
SOUMANA S. , 96  
STANCIU D. , 144  
STANCIU M. , 144  
STEA G. , 98  
STOYANOV K.H. 23, 24, 45, 56  
STROMBERG V. , 17, 18  
SUGIMOTO Y. , 27  
SUZUKI Y. , 75

**T**

TAKEUCHI Y. , 27, 70  
TALEB A. , 54  
TCHELUSTNIKOVA T. , 146  
TEMEL N. , 82  
TETIK O. , 82  
THOIRON S. , 26, 142  
THOROGOOD C.J. , 20  
TIMKO M.P. , 17, 18, 116, 127  
TOSKOVA T. , 102  
TOTH P. , 89  
TOURE A. , 96  
TRAORE H. , 95  
TSVETA H. , 56  
TUINSTRÁ M. , 96  
TURSUN N. , 82  
TÜRKSEVEN S. , 82, 90, 101

**U**

UDUPA S.M. , 128  
Ueda H. , 118  
UENO K. , 76  
ULUDAG A. , 57, 82, 90, 101  
UMEHARA M. , 75  
UYGUR F.N. , 82, 88, 97  
UYGUR S. , 82, 88

**Ü**

ÜREMIŞ İ. , 82  
ÜSTÜNER T. , 82

**V**

VAN AST A. , 96  
VELASCO L. , 145  
VERHEYEN K. , 46  
VERSTAPPEN F. , 68  
VRBNICANIN S. , 73  
VURRO M. , 98

**W**

WALL K. , 17, 18  
WASTERNAK C. , 133  
WEGMANN K. , 47, 117  
WEIK P. , 133  
WESTWOOD J. , 17, 18  
WICKETT N. , 18  
WU B. , 17, 18

**X**

XIE X. , 117

**Y**

YAMAGUCHI S. , 75, 76  
YAZLIK A. , 53, 82  
YILMAZ M. , 143  
YODER J. , 17, 18  
YOKOTA T. , 70  
YONEYAMA K. , 70, 75, 117  
YONEYAMA K. , 27,70,75,117  
YONLI D. , 95  
YOSHIDA S. , 119  
YUE X. , 35  
YÜCEL S. , 82

**Z**

ZAHARAN E. , 64, 113  
ZAROUG M.S. , 64, 113  
ZERMANE N. , 52  
ZIADNA H. , 28, 69  
ZIMDAHL R.L. , 13  
ZOSSOU N. , 38