International Parasitic Plant Society

11th World Congress on Parasitic Plants

7 - 12 June 2011 Martina Franca, Italy

Program and Abstracts

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FOREWORD

Parasitic plants - both the weedy species that severely constrain agriculture and the many other non-weedy species - present unanswered questions with regard to their origin and evolution from non parasitic plants, population structures and dynamics, evolutionary pathways towards crop parasitism, ecology, physiology, molecular biology, and management.

The International Parasitic Plant Congress, now at its 11th edition, continues a long tradition of regularly assembling the world's experts on parasitic plants for professional and scientific meetings, which started in 1973 with the first international meeting in Malta. This year we have a total of 103 abstracts, with 65 oral and 38 poster presentations. We have more than 100 delegates coming from all the continents, with a total of 27 Countries represented.

The Congress brings together scientists representing a wide spectrum of disciplines, research approaches, and geographical representation of parasitic plant research. Assembling specialists with different perspectives, all focused around the common theme of plant parasitism, provides a stimulating environment for learning, exchanging ideas, and connecting with old and new colleagues. The Congress includes presentations at the cutting edge of parasitic plant research and management of parasitic weeds.

Traditional topics related parasitic plants research have been studied since the first congress. These topics include biology, physiology, taxonomy and ecology of parasitic plants, resistance mechanism, breeding, and means for control. During recent years, a increased research has been conducted in parasitic plant genomics and the stimulus of parasitic plant seed germination by strigolactones. These topics have contributed for our understanding of basic questions related to parasitic plant research, therefore in the congress we will focus on these topics in two special symposia: “advances in strigolactone research” and “parasitic plant genomics.”

The Congress' official website is at the following address: **http://ipps2011.ba.cnr.it**

It contains all the information about the congress, and will remain active even after congress' closure to host abstracts and pictures of the event.

ACKNOWLEDGEMENTS

We are very thankful to BASF and Syngenta, for sponsoring the congress.

Thanks to the Cassa di Risparmio di Puglia Foundation, for sponsoring the event.

We are very grateful to the Institute of Sciences of Food Production (ISPA), Italian National Research Council (CNR), Bari, Italy, for supporting the initiative.

Many thanks to the European Weed Research Society, which has provided grants supporting the attendance of 16 young scientists and students from all over the world.

Thanks to the support of the International Institute for Tropical Agriculture (IITA), which made possible the attendance of some scientists belonging to, or collaborating with, that organization.

We are grateful to the Apulia Regional Governorate and the Municipality of Martina Franca for offering their patronage.

Many thanks to the qualified session organizers, who have selected proper oral speakers, and reviewed all the abstracts, guaranteeing a profile highly qualified to the congress

Thanks to the Travel Agency Etleva Viaggi, Bari, who has professionally cared part of the organization.

Many thanks to the qualified staff of the Park Hotel San Michele, Martina Franca, who made possible this enjoyable event.

Hanan Eizenberg, Jim Westwood, Maurizio Vurro

ORGANIZING COMMITTEE

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Session organizers

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YODER John	University of California-Davis, Davis, CA, USA
YONEYAMA Koichi	Utsunomiya University, Utsunomiya, Japan

PROGRAMME AT A GLANCE

7 June - Tue

All day		Arrivals
17:00	20:00	Registration - Poster setup
19:00	22:00	Welcome party

8 June - Wed

07:30	12:00	Registration
08:00	**08:20**	**Opening ceremony**
08:20	**09:20**	**Plenary Lecture: Prof. Koichi Yoneyama**
09:20	09:40	Refreshment break
09:40	**12:40**	**Session 1: Parasitic Plant Genomics**
12:40	14:00	Lunch
14:00	**16:00**	**Session 2: Parasitic Plant Biology**
16:00	16:30	Refreshment break
16:30	**18:30**	**Session 2: Parasitic Plant Biology (continued)**
19:30	21:00	Dinner
21:20		Baroque Concert

9 June - Thu

08:30	**09:25**	**Keynote Lecture: Dr. Hinanit Koltai**
09:25	**10:15**	**Symposium: Advances in Strigolactone Research**
10:15	10:45	Refreshment break
10:45	**12:30**	**Symposium: Advances in Strigolactone Research (continued)**
12:30	14:00	Lunch
14:00	**16:00**	**Session 3: Ecology and Population Biology**
16:00	16:30	Refreshment break
16:30	**18:00**	**Poster Session and Discussion**
18:00	**19:00**	**Student Session**
20:00	23:00	Gala dinner

10 June - Fri

08:00	**10:00**	**Session 4: Host-Parasite Communication**
10:00	10:30	Refreshment break
10:30	**12:30**	**Session 5: Means for Parasitic Weed Management (EWRS session)**
12:30	13:30	Lunch
13:30	20:30	Excursion
20:30		Dinner

11 June - Sat

08:00	**10:00**	**Session 6: Strigolactones**
10:00	10:30	Refreshment break
10:30	**12:30**	**Session 7: Crop Resistance to Parasitic Weeds and Crop Breeding**
12:30	14:00	Lunch
14:00	**15:30**	**Workshop: Using Free Data of the Genome Projects**
15:30	16:00	Refreshment break
16:00	**18:00**	**Session 8 - Interactions Between Parasitic Plants and the Environment**
18:00	**18:30**	**General Discussion - Closure**
18:30		**Student Poster Prize**
20:00		Pizza dinner

12 June - Sun

All day		Departures

DETAILED PROGRAM

7 June - Tue

All day	Arrivals
17:00 - 20:00	Registration - Poster setup
19:00 - 22:00	Welcome party

8 June - Wed

07:30 - 12:00	Registration
08:00 - 08:20	**Opening ceremony**
08:20 - 09:20	**Plenary Lecture - Koichi YONEYAMA** **How many strigolactones do plants produce?**
09:20 - 09:40	Refreshment break
09:40 - 12:40	**Session 1: Parasitic Plant Genomics** **Chairpersons: Jim WESTWOOD and John YODER**
09:40 - 9:45	Jim WESTWOOD Introductory remarks
09:45 - 10:15	Satoko YOSHIDA Large-scale sequencing analysis of *Striga* species
10:15 - 10:45	Jim WESTWOOD and Claude DEPAMPHILIS The parasitic plant genome project: A massive EST sequencing project for the Orobanchaceae
10:45 - 11:05	Julia NAUMANN The *Hydnora* transcriptome project - first genomic insights into the "strangest plant in the world"
11:05 - 11:25	Loren HONAAS Functional genomics of a generalist parasitic plant
11:25 - 11:40	Guangda LIU Horizontal gene transfer between the parasitic plan *Cynomorium songaricum* Rupr. and its host *Nitraria tangutorum* Bobr.
11:40 - 11:55	Gunjune KIM Genomics approaches to understanding mRNA movement between hosts and parasites
11:55 - 12:15	John YODER Parasitic plant genes necessary for haustorium development
12:15 - 12:40	General Session Discussion
12:40 - 14:00	Lunch
14:00 - 16:00	**Session 2: Parasitic Plant Biology** **Chairpersons: Alistair MURDOCH and Gregorio CECCANTINI**
14:00 - 14:15	Erika MAASS Floral biology of *Hydnora abyssinica* – new insights from Southern Namibia
14:15 - 14:30	Golshan ZARE Micromorphological studies on seed of *Orobanche L.*(Orobanchaceae) species from Turkey, and their systematic significance
14:30 - 14:45	Atsushi OZAKAWA Sugar metabolism during germination of *Orobanche minor* as a novel target for selective control
14:45 - 15:00	Alistair MURDOCH Comparison of multiplicative and sequential models of dormancy and germination of *Striga hermonthica*

15:00 - 15:15	Alejandro PÉREZ DE LUQUE Crenate broomrape invasion of pea root: a histological time lapse study
15:15 - 15:30	Mustapha HAIDAR Histological studies on the haustorium of *Cuscuta campestris* Yuncker
15:30 - 15:45	Takeshi FURUHASHI Comparative analysis of seedling proteins of *Cuscuta japonica* attached to different hosts
15:45 - 16:00	Gregorio CECCANTINI Skewed "sex ratios" in the peculiar holoparasite Pilostyles (Apodanthaceae – Cucurbitales)
16:00 - 16:30	Refreshment break
16:30 - 18:30	**Session 2: Parasitic Plant Biology (continued)**
16:30 - 16:45	Anna STĘPOWSKA Morphological response of the tomato *Lycopersicion esculentum* Mill.) to parasitic plants – *Phelipanche ramosa* L. Pomel and pathogen – *Oidium neolycopersici* L. Kiss
16:45 - 17:00	Vitor BARÃO Modifications in wood hydraulic conductivity and embolism increase in *Tipuana tipu* parasitized by *Struthanthus vulgaris*
17:00 - 17:15	James FISHER Redistributing the wealth: interactions between plant parasitism and parasite litter in semi-natural grassland communities
17:15 - 17:30	Sugwang LEE Distribution, characteristics and host specificity of *Loranthus Tanakae* in South Korea
17:30 - 17:45	Daniel NICKRENT Santalales phylogeny prompts new insights into morphological character evolution
17:45 - 18:30	General Session Discussion
19:30 - 21:00	Dinner
21:20	Baroque Concert

9 June - Thu	
08:30 - 09:25	**Keynote Lecture** **Hinanit KOLTAI - Strigolactones' multiple roles in plant development**
09:25 - 10:15	**Symposium: Advances in Strigolactone Research** **Chairpersons: Joseph HERSHENHORN and Hinanit KOLTAI**
09:25 - 09:50	Cristina PRANDI New potent fluorescent analogues of strigolactones: synthesis and biological activity in parasitic weed germination and hyphal branching in AM fungi
09:50 - 10:15	Yoram KAPULNIK Strigolactone substances stimulate different gene expression of tomato light harvesting complexes and hyphal growth of arbuscular mycorrhizal fungi
10:15 - 10:45	Refreshment break
10:45 - 12:30	**Symposium: Advances in Strigolactone Research (continued)**
10:45 - 11:10	Evgenya DOR The synthetic strigolactone GR24 influences the growth pattern of phytopathogenic fungi
11:10 - 11:35	Carolien RUYTER Strigolactones: a cry for help results in fatal attraction. Is any escape possible?
11:35 - 11:50	Hidemitsu NAKAMURA Screening and identification of MAX2-interacting factors for the isolation of novel strigolactone - signaling factors

11:50 - 12:05	Kaori YONEYAMA Interaction between strigolactone and other plant hormones
12:05 - 12:30	General Symposium Discussion
12:30 - 14:00	Lunch
14:00 - 16:00	**Session 3: Ecology and Population Biology** **Chairpersons: Yaakov GOLDWASSER and Garifalia ECONOMOU**
14:00 - 14:15	Mohamed KAMAL Genetic diversity of *Striga hermonthica* populations in Ethiopia: evaluating the role of geography and host specificity in shaping population structure
14:15 - 14:30	Lytton MUSSELMAN The genus *Hydnora* (Hydnoraceae) in Southern Africa and Madagascar
14:30 - 14:45	Leonardo VELASCO Genetic diversity of wild *Orobanche cernua* L. populations from southeastern Spain
14:45 - 15:00	Mat Yunoh SITI-MUNIRAH Distribution of *Rafflesia populations* in Upper Perak, Peninsular Malaysia
15:00 - 15:15	Hans Christian WEBER Observations on parasitic plants of Malta with remarks about the terms root, stem and leaf parasitism
15:15 - 15:30	Peter TÓTH Flower volatiles of Orobanchaceae - a useful tool for phylogeny
15:30 - 16:00	General session discussion
16:00 - 16:30	Refreshment break
16:30 - 18:00	**Poster Session and Discussion** **Chairperson: Jonathan GRESSEL**
18:00 - 19:00	**Student Session**
20:00 - 23:00	Gala dinner

10 June - Fri	
08:00 - 10:00	**Session 4: Host-Parasite Communication** **Chairpersons: Radi ALY and Philippe SIMIER**
08:00 - 08:15	Radi ALY Interactions and translocation of molecules and macromolecules between host plant and broomrape
08:15 - 08:30	Zachary GAUDIN Nitrogen absorption, translocation and fluxes in the *Phelipanche ramosa / Brassica napus* interaction
08:30 - 08:45	Muhammad JAMIL Unraveling the mechanism involved in *Striga* parasitism in cereals under nutrient deficient conditions
08:45 - 09:00	Bruna RODRIGUES-FERREIRA Anatomy of the haustorium of two species of *Struthanthus* Mart. (Loranthaceae)
09:00 - 09:15	Philippe SIMIER Stimulants of *Phelipanche ramosa* germination from oilseed rape roots
09:15 - 09:30	Daniel JOEL The natural germination stimulant of *Orobanche cumana* is not a strigolactone
09:30 - 10:00	General session discussion
10:00 - 10:30	Refreshment break
10:30 - 12:30	**Session 5: Means for Parasitic Weed Management (EWRS session)** **Chairpersons: Diego RUBIALES and Grama Nanjappa DHANAPAL**
10:30 - 10:45	Nadjia ZERMANE Management of broomrape and dodder using natural plant metabolite

10:45 - 11:00	Alistair MURDOCH Could the *Desmodium* 'push-pull' system for *Striga* control in Africa work on *Phelipanche ramosa* and *Orobanche crenata*?
11:00 - 11:15	Alpha KAMARA Integrated management of *Striga hermonthica* in maize in the Nigerian Savannas
11:15 - 11:30	Sarah HEARNE *Striga* the bewitching weed: interdisciplinary context of control
11:30 - 11:45	Hanan EIZENBERG Are we modeling the math or the biology of parasitism dynamics?
11:45 - 12:00	Nuhu GWORGWOR The use of arbuscular mycorrhizal (AM) fungi controlling *Orobanche minor* in red clover (*Trifolium pratense*)
12:00 - 12:30	General session discussion
12:30 - 13:30	Lunch
13:30 - 20:30	Excursion
20:30	Dinner

11 June - Sat

08:00 - 10:00	**Session 6: Strigolactones** **Chairpersons: Koichi YONEYAMA and Antonio EVIDENTE**
08:00 - 08:15	Rodrigo ECHEGOYEN-NAVA How does *Striga hermonthica* alter the growth and morphology of rice plants; are strigolactones involved?
08:15 - 08:30	Kosuke FUKUI Target selective strigolactone analogues
08:30 - 08:45	Juliane Karine ISHIDA Transcriptome analysis of the parasitic plant *Phtheirospermum japonicum*
08:45 - 09:00	Shinsaku ITO Gibberellin regulates strigolactone biosynthesis
09:00 - 09:15	Yukihiro SUGIMOTO Promotive and inhibitory stereoisomers of strigolactones to seed germination of *Striga gesnerioides*
09:15 - 09:30	Binne ZWANENBURG New strigolactone analogues, design, synthesis and bioactivity
09:30 - 10:00	General session discussion
10:00 - 10:30	Refreshment break
10:30 - 12:30	**Session 7: Crop Resistance to Parasitic Weeds and Crop Breeding** **Chairpersons: Julie SCHOLES and Alejandro PÉREZ DE LUQUE**
10:30 - 10:45	Mamadou CISSOKO Post attachment resistance of interspecific NERICA rice cultivars to the parasitic weeds *Striga hermonthica* and *S. Asiatica*
10:45 - 11:00	Haron KARAYA Combining ability of maize inbred lines resistant to *Striga hermonthica* evaluated under artificial *Striga* infestation in Kenya
11:00 - 11:15	Johann LOUARN Can we use arbuscular mycorrhizal fungi to improve resistance to *Orobanche cumana* in sunflower?

11:15 - 11:30	Joseph HERSHENHORN Characterisation of a novel tomato mutant HRT-1 resistant to acetolactate synthase inhibiting herbicides for broomrape management
11:30 - 11:45	Leonardo VELASCO Studies on plant isolation and hybridisation in sunflower broomrape (*Orobanche cumana* Wallr.).
11:45 - 12:00	Yaacov GOLDWASSER Differential susceptibility of pepper (*Capsicum* spp.) to *Phelipanche aegyptiaca*
12:00 - 12:30	General session discussion
12:30 - 14:00	Lunch
14:00 - 15:30	**Workshop: Using Free Data of the Genome Projects**
15:30 - 16:00	Refreshment break
16:00 - 18:00	**Session 8 - Interactions Between Parasitic Plants and the Environment** **Chairpersons: Hanan EIZENBERG and Ahmet ULUDAG**
16:00 - 16:15	Sebastian BÖKLE Influence of fertilization and field history on soil properties and microbial communities and its' relation to *Striga hermonthica* (Del.) Benth. population density, in the Kati district of Mali
16:15 - 16:30	Amnon CHOCHAVI Developing a predictive model based on temperatures for *Phelipanche aegyptiaca* parasitism in carrots
16:30 - 16:45	Garifalia ECONOMOU A large scale analysis of factors affecting the infestation of tobacco (*Nicotiana tabaccum* l.) by *Phelipanche* species
16:45 - 17:00	Jonne RODENBURG Preparing African rice farmers against parasitic weeds in a changing environment – a new, integrated research project
17:00 - 17:15	Ahmet ULUDAG Understanding climate change on parasitic plants' invasions
17:15 - 17:30	Tuvia YACOOBY Malvaceae weeds as hosts for *Orobanche aegyptiaca* in Israel
17:30 - 18:00	General session discussion
18:00 - 18:30	**General Discussion - Closure**
18:30	**Student Poster Prize - Chairperson: Julie SCHOLES**
20:00	Pizza dinner

12 June - Sun

All day	Departures

ABSTRACTS

HOW MANY STRIGOLACTONES DO PLANTS PRODUCE?

Xie X., Kisugi T., Yoneyama K., Nomura T., **Yoneyama K.**

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After the first characterization of strigol and strigyl acetate as germination stimulants for the root parasitic weed *Striga lutea* (syn. *S. asiatica*), more than 10 compounds belonging to the same chemical class called strigolactones (SLs) have been identified. Recent advances in purification and analytical methods along with those in spectroscopic techniques have enabled isolation and structural determination of chemically unstable SLs present only in trace amounts. All of natural SLs were isolated from plant root exudates as germination stimulants except for 5-deoxystrigol, which was originally identified as a branching factor, an essential signal for root colonization by symbiotic arbuscular mycorrhizal (AM) fungi. In addition to these functions in rhizosphere, it has been shown that SLs or their metabolites are a novel class of plant hormones regulating plant aboveground architecture. Furthermore, SLs are suggested to have other biological functions in rhizosphere communications and in plant growth and development, e.g., light perception and root system architecture.

Since all angiosperms so far examined produce and release SLs into the rhizosphere, any organisms in the vicinity of plant roots are continuously exposed to SLs, indicating that SLs have profound effects on the chemical communications between the SL-producing plants and the other organisms in the rhizosphere. Not only the vascular plants but also non-vascular plants mosses (*Physcomitrella patens* and *Marchantia polymorpha*) produce SLs, suggesting that SLs existed when plants first colonized lands, and thus soil organisms have been exposed to SLs from the very beginning of the history of land plants.

These natural SLs are composed with a tricyclic lactone (ABC part) that connects via an enol ether bridge to a butenolide group (D ring). The enol ether bridge is regarded as the essential structure for germination stimulation activity and indeed all of the natural SLs contain it. Structural modifications in the other parts of the molecule also affect the activity and stability and their effects may be different with each root parasite.

Natural SLs contain 3 to 5 asymmetric carbons and therefore consist of 4 to 16 stereoisomers; 4 stereoisomers for the simplest SL, 5-deoxystrigol and 16 stereoisomers for 7-hydroxyorobanchol. The identification of *ent*-SLs such as *ent*-2′-epi-5-deoxystrigol, *ent*-2′-epiorobanchol, and solanacol, suggests that plants may produce all stereoisomers and only those produced at larger amounts or accumulated have been detected so far. These stereoisomers may be acetylated or conjugation with sugars or amino acids may occur. Accordingly, it is likely that plants produce more than 100 SLs including conjugates and stereoisomers.

Session 1

PARASITIC PLANT GENOMICS

Chair

Jim WESTWOOD and John YODER

PARASITIC PLANT GENES NECESSARY FOR HAUSTORIUM DEVELOPMENT

Bandaranayake P.C.G., Filappova T, Tomilov A., Tomilova N., **Yoder J.I.**

Department of Plant Sciences, University of California-Davis, Davis, CA USA

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Parasitic Orobanchaceae use chemical and tactile stimuli provided by host roots to initiate the development of invasive haustoria. We are interested in discovering the molecular events associated with host signal perception and transduction in order to identify potential parasite gene targets for engineering weed resistant crops. On the basis of our participation in the Parasitic Plant Genome Project as well as earlier studies, we have sequenced thousands of mRNA transcripts expressed in roots of the hemiparasite *Triphysaria* during host signal recognition and early haustorium development. Because of the potential role of redox signaling in rhizosphere interactions between plants, we initially focused on two genes that encode quinone oxidoreductases and which are transcriptionally regulated in parasite roots by host roots or host root factors. The enzymes encoded by both genes catalyze the NAD(P)H dependent reduction of haustorium inducing quinones but through different mechanisms. The TvQR1 enzyme catalyzes single electron quinone reductions that produce unstable, radical semiquinones and, in aerobic environments, consequential reactive oxygen species. In contrast, TvQR2 catalyzes two electron reductions that bypass the potentially toxic semiquinone intermediates. We made hairpin RNA silencing constructions targeting either TvQR1 or TvQR2 and transformed these into *Triphysaria* roots using *Agrobacterium rhizogenes*. Silencing of TvQR1 in *Triphysaria* results in a dramatic decrease in the number of haustoria produced while silencing TvQR2 has no effect on haustorium development. We propose that TvQR1 catalyzes a univalent reduction of host molecules that activates a redox sensitive transduction pathway that signals haustorium development. The toxic reactive oxygen species produced by TvQR1 are detoxified by the action of TvQR2. Our model proposes that the balance between TvQR1 catalyzed semiquinone production and TvQR2 mediated semiquinone elimination describes the cellular environment for haustorium initiation. The transcriptional up-regulation of TvQR1 in response to host root contact is interesting because the homologous genes are down-regulated in *Arabidopsis* and other non parasitic species exposed to the same conditions. Current work is directed towards understanding the transcriptional regulation of TvQR1 with the goal of identifying *cis* and *trans* acting factors that regulate parasite specific gene regulation.

THE PARASITIC PLANT GENOME PROJECT: A MASSIVE EST SEQUENCING PROJECT FOR THE OROBANCHACEAE

dePamphilis C.[2], Wickett N.[2], Fernández-Aparicio M.[1], Das M.[1], Huang K.[3], Wu B.[4], Zhang Y.[2], Honaas L.[2], Alford S.[1], Landherr L.[2], Stromberg V.[1], Timko M.[3], Yoder J.[4], **Westwood J.**[1]

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The Parasitic Plant Genome Project is taking a comparative evolutionary approach to understanding parasitism in the Orobanchaceae. The project is sequencing expressed sequence tags (ESTs) from three species, a facultative parasite (*Triphysaria versicolor*), a photosynthetically competent obligate parasite (*Striga hermonthica)*, and an obligate holoparasite *(Orobanche aegyptiaca)*. In addition, ESTs have been sequenced from the basal, nonparasitic Orobanchaceae species *Lindenbergia philippensis*. To date, we have generated a total of more than 1.3 billion 454 and Illumina reads, and from this have assembled approximately 50,000 unigenes from each of the three focal parasites, representing all life stages from seed conditioning to flowering. Availability of the full genome of the non-parasitic relative, *Mimulus guttatus*, provides additional power to discover the genome-wide changes associated with the parasitic lifestyle. The sequences are provided as a resource to the public in downloadable form and a searchable database at http://ppgp.huck.psu.edu/. Results of data analysis to date indicate that alterations in gene expression have been important in the evolution of parasitism, but there are also examples of gene gain (through individual gene duplications and polyploidy) and loss. Despite the striking morphological differences between *Triphysaria*, *Striga*, and *Orobanche*, the numbers and types of genes expressed in each of these species are similar, with the notable exception of genes associated with photosynthesis. We detected no evidence of expression of genes for proteins involved in light harvesting or photosynthetic electron transport in *O. aegyptiaca*; genomic sequencing will be required to discover whether this is due to large scale suppression of gene expression or to pseudogenization and loss of the genes. In sharp contrast, there is strong evidence for the retention of genes encoding proteins in the chlorophyll biosynthetic pathway in *O. aegyptiaca*, a surprising result in the face of the widely held belief that these parasites are completely lacking in chlorophyll. Another example of surprising expression of genes is the identification of all known genes associated with the synthesis of strigolactones in *Striga* and *Orobanche*, raising the likelihood that parasites synthesize their own strigolactones. Other lines of investigation have revealed the presence of several cases of horizontal gene transfer of nuclear-encoded host genes to parasites. In one case, a legume gene appears to have moved into *Orobanche* and encodes a protein with potential value in insect resistance. These are just a few of the ways in which this project accelerates the pace of research in parasite evolution and function.

FUNCTIONAL GENOMICS OF A GENERALIST PARASITIC PLANT

Honaas L.[1], Wickett N.[1], Wafula E.[1], Zhang Y.[1], Der J.[1], Altman N.[2], Bao Y.[3], McCombie W.[4], Schuster S.[5], Taylor C.[6], Yoder J.[7], Landherr L.[1], Wu B.[7], dePamphilis C.[1]

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The Orobanchaceae are a plant family whose parasitic members growing in Africa and the Mediterranean include the devastating agricultural pests, witchweed (*Striga*) and broomrape (*Orobanche*). In addition to the pressing socioeconomic issues surrounding these parasites, this family represents a unique opportunity to study parasite biology since its members span varying degrees of parasitism from feel-living to non-photosynthetic, obligate parasites. The Parasitic Plant Genome Project (PPGP, http://ppgp.huck.psu.edu/) aims to discover the genetic changes in Orobanchaceae that allowed the transition from autotrophy to heterotrophy by transcriptome analysis of various tissue and life stage-specific samples. Next Generation Sequencing (NGS) technology is allowing the discovery of genes being used by the parasite in each specific sample and builds a comparative framework that will facilitate identification of genes involved in the parasitic lifestyle. Despite the great power of this approach, gene expression patterns of highly specialized cells can become diluted since they may represent a small percentage of the total tissue harvest. We are using laser capture micro-dissection to sample tissues in a highly cell-specific manner, extract and then amplify exceedingly small RNA samples, sequence them with NGS technology and analyze them without the aid of prior sequence knowledge. We are guiding our efforts of de novo assembly and subsequent gene expression analysis by analyzing the young leaf transcriptome of the sequenced model plant, *Arabidopsis thaliana*. Applying the above described methods we analyzed the host-parasite interface transcriptomes of a generalist member of the Orobanchaceae, *Triphysaria versicolor*, grown on the distantly related model hosts *Zea*

GENOMICS APPROACHES TO UNDERSTANDING mRNA MOVEMENT BETWEEN HOSTS AND PARASITES

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Cuscuta pentagona (lespedeza dodder) takes up mRNAs from its host plants. Previous research has suggested that there are tens, and may be hundreds of host mRNAs that are trafficked into the parasite, but nothing is known about the mechanisms regulating cross-species transfer or its biological significance to the parasite. We addressed this phenomenon with a genomics approach that used Illumina sequencing to assess the presence of host mRNAs in the parasite and potentially parasite mRNA in the host. *Cuscuta* was grown on two hosts, *Arabidopsis* and tomato, in order to compare mRNA uptake from different species. Furthermore, the use of well-sequenced host plants facilitates bioinformatics identification of host transcripts that have moved into dodder. From each host-parasite interaction three regions of tissue were harvested: the *Cuscuta* alone, the region of *Cuscuta* attachment on the host, and the host stem just above the region of attachment. Approximately 30 million paired-end reads of 75bp in length were generated from each of the six tissues in hosts and parasite system. By mapping reads against known host sequences we were able to identify host mRNAs in *Cuscuta* and determined that a large proportion of the host transcriptomes are trafficked into the parasite. We detected 11,248 possible mobile *Arabidopsis* RNAs and 3,301 mobile tomato RNAs, representing approximately one-half and one-twelfth, respectively, of the genes expressed in the host stems. We also found evidence for bidirectional movement of mRNAs between host and parasite, identifying 616 *Cuscuta* RNAs in stem tissue of both hosts. The mobile RNAs from both hosts and parasite were functionally categorized using GO-slim terms. At this level, the classes of genes identified as mobile were broadly representative of overall plant metabolism. Detailed investigation of mobile mRNAs are consistent with other reports of phloem-mobile mRNAs as expected of a phloem-feeding parasite. We expect that further comparative genomics analyses will provide insight into mechanisms of mRNA mobility and parasite biology.

DIFFERENTIAL GENE EXPRESSION IN *CUSCUTA REFLEXA*

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Cuscuta, *Phelipanche* and *Striga* are different parasitic plants, which rely on host-derived carbohydrates and nutrients. To obtain these substances the parasite connects its own phloem and xylem with the host vascular tissue via special organs, so called haustoria. The development of haustoria may be affected by various gene products of the parasite and/or the host. To find more of these gene products differential gene expression in *Cuscuta reflexa* shoots with and without haustoria was examined by using cDNA AFLP (Amplified Fragment Length Polymorphism). After gel analysis approximately 150 distinct bands were excised. Now they are being cloned and sequenced step-by-step. Until now various gene products have been identified by comparison with other known sequences in databases like NCBI. The most promising gene products and their corresponding genes will be further investigated with regard to their function and their occurrence in species other than *Cuscuta reflexa*. Especially the latter can provide more general strategies in the battle against parasitic plants.

IDENTIFICATION AND DISTRIBUTION OF HOST mRNA TRANSCRIPTS IN THE PARASITIC PLANT DODDER

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Lespedeza dodder (*Cuscuta pentagona* Engelm.) absorbs mRNA from parasitized tomato (*Lycopersicon esculentum* Mill.). Initial work using microarray analyses revealed 474 putatively mobile transcripts, 21 of which have confirmed host-to-parasite mobility. The exact mechanism of uptake and utility of host mRNA in the parasite is not well characterized. We have begun to describe the patterns of mRNA taken up as well as its distribution in the parasite. Real Time Quantitative PCR (RT-qPCR) experiments using the tomato transcription factor *Giberellic Acid Insensitive (LeGAI)* showed that copies of the mRNA signal were orders of magnitude lower in the parasite compared to the host and in decreasing concentration in the parasite as the distance from the host increased. In contrast to *LeGAI*, the tomato *Cathepsin D Proteinase Inhibitor (LePI)* transcript, which is inducibly expressed by the host at the site of parasitic attack to counteract an aspartyl protease, showed a different degree of detection in the parasite. RT-qPCR indicated that dodder contains high levels of host PI mRNA, with transcript copies in dodder present at just half the numbers as detected in the host stem. *LePI* mRNA in dodder also showed more even distribution around the point of attachment and towards the tip of the dodder. A recent transcriptome sequencing experiment looking at parasitized host, dodder and attachment site tissues has confirmed these observations. While no mapped *LeGAI* transcripts were detected in the parasite growing on tomato, 2 mapped copies of a homolog were found in dodder growing on *Arabidopsis*. The more abundant *LePI* had 115 mapped reads found in dodder on tomato. These results demonstrate that host mRNAs in dodder are either acquired via different mechanisms that allow selective filtering, or differ in stability such that some mRNAs persist in higher abundance than others.

HORIZONTAL GENE TRANSFER BETWEEN THE PARASITIC PLANT *CYNOMORIUM SONGARICUM* RUPR. AND ITS HOST *NITRARIA TANGUTORUM* BOBR.

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Horizontal gene transfer (HGT) is the transfer of genes between non-mating species; parasitic relationship may facilitate HGT between flowering plants, but mechanistic explanations for HGTs have remained speculative. In this research, we report the discovery of *atp1* gene HGT from host *Nitraria tangutorum* (Bobr.) to its obligate parasitic plant *Cynomorium songaricum* (Rupr.). *Cynomorium* has two species, *C. coccineum* of northern Africa and the Mediterranean region and *C. songaricum* of western Asia, the latter species, known as "suo yang" in Chinese. The hosts of *C. coccineum* mainly belong to Caryophyllales and Asterales and no host of the Sapindales has been discovered. The host of *C. songaricum* is *N. tangutorum* in China, which belongs to Sapindales. We amplified and sequenced *atp1*, *cox1*, *matR*, *18srDNA* genes of *C. songaricum* and its host *N. tangutorum*, compared the sequences with the homologous genes of *C. coccineum* and other plants. Phylogenetic trees were estimated based on ML, MP, NJ, Bayesian analysis. Phylogenetic analyses shows that *atp1* places *C. coccineum*, *C. songaricum* and *N. tangutorum* in Sapindales. However, according to the phylogenetic analyses of *cox1*, *matR* and *18srDNA*, *C. coccineum* and *C. songaricum* are not placed in Sapindales, which suggests HGTs might have happened between the common ancester of Cynomorium and Sapindales. We also found that *atp1* of *C. songaricum* shows the character of chimerism, a 720bp fragment of the *atp1* gene of *Cynomorium songaricum* shows high similarity to *N. tangutorum*, even higher than *C. coccineum*. This suggests that after the divergence of *C. coccineum* and *C. songaricum*, a part of the *atp1* gene was horizontally transferred between *C. songaricum* Rupr. and *N. tangutorum*. In this 720bp fragment, there are 10 bases different between *C. songaricum* and *C. coccineum*, but there is only 1 amino acid different, and it's an Arginine to Lysine substitution. These two amino acids have very similar properties, they are both basic amino acids and hydrophilic amino acids. This suggests that the gene fragment of *atp1* horizontally transferred may not affect the function of the protein. The results of this research suggest there were HGT between the common ancestor of Cynomorium and Sapindales. After the divergence of *C. coccineum* and *C. songaricum*, HGT still happened between *C. songaricum* and *N. tangutorum*. Although *C. songaricum* and *C. coccineum* belong to the same genus, some genes will co-evolve with the different hosts they chose.

THE *HYDNORA* TRANSCRIPTOME PROJECT - FIRST GENOMIC INSIGHTS INTO THE "STRANGEST PLANT IN THE WORLD"

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The holoparasitic Hydnoraceae have been called the "strangest plants in the world" for numerous reasons. Their highly modified morphology as well as the absence of most or all plastid gene markers left their phylogenetic position unclear for many years. In 2002, mitochondrial markers revealed that Hydnoraceae are basal angiosperms and placed together with Aristolochiaceae within Piperales. Thus, from an evolutionary point of view, Hydnoraceae are the most basal branch of angiosperms that has adopted the holoparasitic lifestyle and become entirely dependent on a host plant. We performed a pilot study of the *Hydnora* transcriptome (Illumina paired end sequencing) that has given a first glimpse of the expressed gene set of this remarkable organism. We extracted single copy nuclear genes (nSCG) of *Hydnora visseri* from this data. In combination with Sanger and NGS ESTs of representatives of basal angiosperm families (Piperaceae, Aristolochiaceae, Lactoridaceae, Saururaceae, Hernandiaceae, Monimiaceae, Lauraceae, Calycanthaceae, Annonaceae, Magnoliaceae, Chloranthaceae, Canellaceae, Nymphaeaceae, Amborellaceae) the phylogenetic trees resolve Hydnoraceae as member of Piperales and closer to *Aristolochia* than two other genera such as *Asarum* or *Saruma*. Reconstruction of the phylogenetic hypothesis is based on 13 nuclear genes using both Maximum Likelihood and Bayesian analyses. This phylogeny broadly covers both monocots and eudicots as well, and thus a strong framework is provided. In the second part of the talk we will present preliminary insights into the possible occurrence of horizontal gene transfer (HGT) between *Hydnora* visseri and its host *Euphorbia gregaria* in southern Africa. An initial 3-step screening was performed using *Hydnora* transcriptome data and ESTs from basal angiosperms representing the parasitic side. For the host, several Fabaceae and Euphorbiaceae ESTs were used. This screening identified 25 genes that were potentially transferred from the host to the parasite's genome, which will be investigated in more detail in the near future.

LARGE-SCALE SEQUENCING ANALYSIS OF *STRIGA* SPECIES

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Striga spp. (common name witchweeds) are noxious parasitic plants which infest major crops and cause considerable yield losses in sub-Saharan Africa and parts of Asia. Understanding the molecular basis of parasitism will contribute to develop control methods of those parasites. However, the molecular and genomic resources of *Striga* species are still limited. To obtain sequence information of functional genes in *Striga*, we performed a large-scale expressed sequence tag (EST) analysis of *Striga hermonthica*. A full-length enriched cDNA library was constructed using RNAs from various tissues and developmental stages of *S. hermonthica* as materials. We sequenced over 35,000 clones from both ends and the sequences were assembled into 17,317 non-redundant unigenes that included 10,319 contigs and 6,818 singletons. We found 1,445 simple sequence repeats (SSRs) in the *Striga* unigenes. Those SSRs can be used as genetic markers to investigate genetic diversity of *S. hermonthica* in Africa. Comparative analysis of *S. hermonthica* ESTs and other plant genomes found evidence for horizontal gene transfer of a nuclear-encoded gene from a host to a parasite. The eudicot *S. hermonthica* parasitizes only Poaceae species. However, we found that a *S. hermonthica* nuclear-encoded gene, designated *ShContig9483*, is widely conserved in Poaceae species but not found in other eudicot species. *ShContig9483* is clustered with genes from sorghum, a natural host of *S. hermonthica*, suggesting that the S. hermonthica acquired the gene from a host through horizontal gene transfer. High similarity between *ShContig9483* and its sorghum homologs in the coding region as well as the 5' and 3' untranslated regions indicates the transfer was relatively recent. Aiming to understand complete picture of *Striga* evolution and molecular mechanisms of its parasitism, we recently started a *Striga* genome project. Using next generation sequencers, the *Striga* genome will be sequenced and assembled. The genome resource from *Striga* will bring new insights to parasitic plant studies.

Session 2

PARASITIC PLANT BIOLOGY

Chair

Alistair MURDOCH and Gregorio CECCANTINI

MODIFICATIONS IN WOOD HYDRAULIC CONDUCTIVITY AND EMBOLISM INCREASE IN *TIPUANA TIPU* PARASITIZED BY *STRUTHANTHUS VULGARIS*

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Leaves of hemi-parasitic plants represent extra transpiring area to their hosts. Many of these plants have very low hydraulic potential and lose water to the atmosphere in large proportions. Once they are connected to the host xylem, hemi-parasitic plants may therefore compete for water with the crown of their host and modify its hydraulic system. The aim of this work was to evaluate xylem anatomy and water conduction of *Tipuana tipu*, parasitized by *Struthanthus vulgaris*, in a morpho-functional approach. Parasitized and non-parasitized branches were collected, cut under water and surfaced with sharp blades. They were then carefully transferred to Safranin solution (0,1%) avoiding new embolism formation, and allowed to transpire. In wood anatomical analysis, stained vessels were considered as conductive and the non-stained ones as non-functional and embolized. The embolism frequency was evaluated by the non-stained vessel proportion. The vessels had their frequency and area measured and their individual hydraulic conductivity mathematically estimated. Also the specific hydraulic conductivity was calculated (by area) for the two outermost rings, which can be assumed to have been formed after parasite infection. The measurement of vessel area and frequency was performed by an automatic image analysis adjusted by manual correction. To detect morphological and functional effects of parasitism, the variables, vessel frequency, specific hydraulic conductivity for total wood and for functional vessels only, were compared between parasitized and non-parasitized branches. Significant changes were observed in the frequency of embolized vessels, with parasitized branches showing about twice the frequency found in non-parasitized ones. The average specific hydraulic conductivity of parasitized branches was around 7.6×10^{-7} $Kg.m^{-1}.MPa^{-1}.s^{-1}$ with about double the loss of theoretical conductivity caused by embolism, when compared to the non-parasitized wood. These results show the systemic functional impact of parasitism in branches, considering that *S. vulgaris* haustoria are distributed along large extensions of the branch axis, due to the presence of long epicortical roots with many holdfasts.

SKEWED "SEX RATIOS" IN THE PECULIAR HOLOPARASITE *PILOSTYLES* (APODANTHACEAE – CUCURBITALES)

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The Apodanthaceae are holoparasitic plants with extreme vegetative reduction. Their vegetative body does not show traditional organs, bearing only an endophyte, formed by parenchymatic cell masses, inside host tissues. The only evidence of their presence is the eruption of buds and flowers from host epidermis or peridermis. They are very specialized; with staminate flowers bearing an adroecium in a synandrium, with two to four pollen sac rings attached to a central column. Pistillate flowers have inferous tetracarpelar unilocular ovarium. Their spority has been much debated, because it is almost impossible to distinguish one parasite from other when they occur in the same host. Authors accept the dioecious condition, but they refer to the old circumscription of Rafflesiaceae (s.l.). So this work aimed to understand if the "sex ratio" is controlled by the parasite, by its hosts or by geographic factors. So, we surveyed four populations in Brazil and Mexico, countries with the highest diversities of *Pilostyles: P. ulei*, hosted by *Mimosa foliolosa*, from Serra do Cipó, Minas Gerais State, Brazil; *P. thurberi*, hosted by *Dalea bicolor* from El Cervo e Los Trigos, both from Queretaro State, and hosted by *Dalea melantha* var *melantha*, from Santa Isabel, Puebla State, Mexico. We collected and fixed 50-100 flowers, from 10-100 hosts, depending on the local availability. Later they were carefully dissected under stereomicroscope, to determinate if they were pistillate or staminate, and calculated the "sex ratio" or "spority ratio" of each population. Flowers were also prepared by standard histological methods. For the first time in this family the presence of monoclinous flowers was identified. *M. foliolosa* from Serra do Cipó showed 83% hosts with pistillate (P) and staminate (S) flowers together and 17% only with pistillate. *P. thurberi* showed three distinct behaviors: in El Cervo each host had only one flower type (S60:P40); in Los Trigos hosts with both flowers (11.9%) and hosts with separated flowers (S44:P56); Santa Isabel 100% with both flowers. In *P. ulei*, among hosts with both flower types staminate flowers number is very smaller than pistillate number (5.9:1). Counting all individuals the sex ratio is 7.9:1, so there are 7.9 pistillate flowers to each staminate one. For *P. thurberi* the ratio was 0.8:1 in Los Trigos and 0.6:1 in El Cervo but very different in Santa Isabel 2.3: 1, with domination of pistillate flowers. For *P. ulei* monoclinous flowers are rare, with less than 1.3% of the total, but occurring in 49% of hosts, what is similar for *P. thurberi*, but only in hosts with staminate flowers only. So it is clear that *Pilostyles* can exhibit its sexual expression in each population, so, probably the flower morphogenesis is not under a strict or severe gene control, but is very plastic and suitable of influences of each host and unknown environmental effects.

CRENATE BROOMRAPE INVASION OF PEA ROOT: A HISTOLOGICAL TIME-LAPSE STUDY

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There are several papers dealing with the infection process of parasitic weeds in crops. However, still great gaps remain in our knowledge about the initial stages of the pathogenesis. The invasion of the tissues is the acid test for the parasite, because an intimate contact with the host will occur and natural barriers to penetration (even in suitable and susceptible host) are expected. The pathogen must display a wide array of 'tools' to overcome the intrinsic resistance present in a compatible host. In addition, an obligate holoparasitic plant such as broomrape must connect with the vascular system of the host during the short period of time that its independent phase lasts. There are no detailed studies about the time course of the penetration process by broomrape on susceptible hosts, but most of the works assume a time course of approximately one week. However, due to the increasing accuracy of new analytical methods and instrumentation, a more precise knowledge about the invasive steps is required. We have used histological techniques in order to follow the penetration process of *Orobanche crenata* in susceptible pea (*Pisum sativum*). The results have shown that at early stages (up to 3 days after inoculation (DAI) of the pea roots with germinated broomrape seedlings) no penetration of the host tissues was detected. Only at 4 DAI some initial stages of invasion of the host cortex were observed, and 11 DAI the parasite intrusive cells reached the endodermis and the central cylinder. Vascular connections with the host were established 12 DAI. It is clear that when collecting samples for experiments involving gene expression analysis, the time of collection is a key issue. The results will be different if samples are taken 6 DAI when the parasite still remains in the cortex, compared to samples collected 12 DAI, when the parasite has crossed the endodermis invading the central cylinder. Despite differences found regarding individual broomrape seedlings, a correlation between time and the infection stage within the host tissues can be established.

COMPARISON OF MULTIPLICATIVE AND SEQUENTIAL MODELS OF DORMANCY AND GERMINATION OF *STRIGA HERMONTHICA*

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Despite the tremendous amount of research on *Striga hermonthica*, quantitative relationships of the dormancy and germination responses of the parasite to key environmental factors have not been transcribed into mathematical models in the way achieved for *Orobanche* species. However, a pre-requisite to modelling the dual processes of loss of primary dormancy and the induction of secondary ("wet") dormancy and the consequent germination responses of *S. hermonthica* seeds depends on the selection of a suitable model with biologically meaningful parameters. Estimates of such parameters can then be used to describe and compare germination responses to important environmental factors such as water stress, temperature and fertility. Previous models on *Orobanche* have assumed that loss of primary dormancy and induction of secondary dormancy are independent and concurrent processes. The hypothesis of independence and concurrence was never tested against an alternative that the two processes are dependent and sequential.

In this paper, three multiplicative and four sequential models were evaluated to test these hypotheses. The models also address two other key questions unanswered in many previous models. First of all, do models need to include a "control dormancy" or active fraction of seed population analogous to "control mortality" studies using probit analysis? Secondly, is there a lag period between the start of conditioning and the onset of induction of secondary dormancy? The seven models were initially evaluated on two treatments. Among the models evaluated, a multiplicative probability model gave the least residual deviance, but could not be considered as the best model on account of the inconsistent estimation of the lag periods across forty environments leading to acceptance of an additive model and of the dependent, sequential hypothesis.

REDISTRIBUTING THE WEALTH: INTERACTIONS BETWEEN PLANT PARASITISM AND PARASITE LITTER IN SEMI-NATURAL GRASSLAND COMMUNITIES

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It has long been accepted that parasitic plants can act as keystone species and may cause dramatic changes to the communities which they infect. These shifts in community composition partly result from the direct impact of the parasite on susceptible hosts, through the impairment of host growth and metabolic performance. However, parasitic plants may also be able to mould their host communities through indirect means, for example by altering the biogeochemical cycling of nutrients through the ecosystems in which they occur. *Rhinanthus minor* is an annual root-hemiparasite known to induce dramatic shifts in the grassland communities that it inhabits. The parasite suppresses the biomass of grasses within a community, consequently allowing forbs (non-leguminous dicots) to proliferate. This shift in community composition is underpinned by the differential ability of host species to resist the parasite. In general forbs are able to resist the invading parasite strongly while grasses are unable to mount an effective resistance response. In addition to this direct impact, *R. minor* may also influence the structure of its host community by altering the path of nutrients through grassland ecosystems. Specifically, *R. minor* may extract nutrients from its perennial hosts and return them to the soil via its nutrient rich litter. However, which group benefits from this increased flux of nutrients into the soil? Do these liberated nutrients act to ameliorate the direct impact of parasitism on susceptible hosts or further benefit the resistant ones? Do all species or functional groups within a grassland community benefit equally from the *R. minor* induced nutrient flush? Initial results from mesocosm studies at the University of Sheffield have demonstrated that nutrient rich *R. minor* litter can partially ameliorate the direct impact of the parasite on the grasses but that forbs may also benefit from litter input when the parasite is present. However, can these trends derived from highly controlled model communities be observed in the field? And what is the impact of *R. minor* parasitism and litter, in combination and singularly, on the availability of nutrients in the soil? In 2005 a field experiment was established in Derbyshire, UK to address these questions. Four treatments applied were (i) infection by *R. minor*, (ii) treatment with *R. minor* litter, (iii) infection by *R. minor* plus litter treatment and (iv) control in 40 1.25x1.25m plots. In August 2009 and 2010 vegetation surveys were undertaken and followed an above-ground harvest. The biomass, tissue N and tissue P were determined for each functional group within each plot. Plant available N and P in the soil was also determined at bimonthly intervals from October 2009 until August 2010. The vegetation surveys revealed a clear impact of *R. minor* infection on community composition; although the effect of parasite litter on these communities was less clear at the species level. However, at the functional group level, the first evidence that parasitic plant litter can have different effects on discrete functional groups within natural plant communities in the field, is shown. Furthermore, the soil chemistry assays demonstrated for the first time that parasitic plant litter can have an impact on the availability of N in the soil. Thus to understand the role that parasitic plants play in modulating plant community structures, it is necessary to consider both their direct parasitic effects and their indirect impacts on nutrient cycling.

COMPARATIVE ANALYSIS OF SEEDLING PROTEINS OF *CUSCUTA JAPONICA* ATTACHED TO DIFFERENT HOSTS

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The stem holoparasitic plant, *Cuscuta*, causes serious problems in crops. Although some research on this plant has been done previously, proteomics analysis of *Cuscuta* attached to different host plants has not been investigated.
In this study, proteomic analyses based on LC/MS and following MAPA (mass accuracy precursor alignment) were applied. Proteins were extracted from *Cuscuta japonica* seedlings, which were either unattached or else attached to host plants. Principal component analysis (PCA) showed that seedlings treated with a FR light and contact signal were categorized into similar groups. Seedlings without any cue are rather similar to haustorium induced seedlings. Haustorium-induced *Cuscuta* seedlings without host plants were not clearly separated from *Cuscuta* attached to *Buxus*, *Conyza* or *Pueraria*.

ION FLUX KINETICS IN BLUE-LIGHT GROWN DODDER (*CUSCUTA* SPP.) SEEDLINGS

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Previous studies revealed that blue light (BL) stimulates coiling and prehaustorial development in young de-etiolated dodder seedlings prior to host attachment. In this study, Ca^{2+} and H^+ flux kinetics associated with BL mediated coiling and prehaustorial development in de-etiolated dodder seedlings were measured non-invasively using ion-selective vibrating microelectrodes (the MIFE technique). Findings show that BL induces influxes of Ca^{2+} and caused a decrease in apparent net H^+ efflux. A clearly pronounced lag of about 2.0 min was observed between BL-induced changes in Ca^{2+} and H^+ fluxes. Addition of vanadate (100μM) to the bathing solution completely prevented Ca^{2+} and H^+ fluxes responses and inhibited coiling and prehaustorial development, suggesting involvement of Ca^{2+} and H^+ in BL-induced responses of dodder. Information about BL-mediated ionic mechanisms affecting dodder's parasitic mode of growth significantly advances our understanding of light signal transduction models and the role that signal transduction plays in "fine-tuning" a plant's response to its environment.

DISTRIBUTION, CHARACTERISTICS AND HOST SPECIFICITY OF *LORANTHUS TANAKAE* IN SOUTH KOREA

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The mistletoes are represented by two families, four genera and five species in Korea (Loranthaceae and Santalaceae). *Loranthus tanakae* occurs in Korea, China and Japan. *L. tanakae* is a deciduous, hemiparasitic shrub which grows to a height of 1 m and is similar to L. europaeus. *L. tanakae* is an endangered species in Korea, since it has been used in traditional medicine and is a host-specialist (<10 host species).

The objectives of this study were to clarify the distribution of *L. tanakae*, to evaluate the degree of infestation and also its relationships to hosts and host specificity. Forty sites (1km 1km) were sampled in Gangwon Province, Korea. A total of 420 host individuals were examined with measurements of the diameter at breast height (DBH) and heights of parasitism. One thousand, two hundred and seventy *L. tanakae* plants were found on the 420 host trees with 665 *Viscum album* var. *coloratum* on 227 of the trees. *L. tanakae* was distributed only in the natural forest while *V. album* var. *coloratum* was distributed not only in natural forest but also in planted areas. The infested host trees belonged to nine species from six genera and five families. Of total number of individuals of *L. tanakae* 86.9% were growing on Fagaceae, 10% on Betulaceae, 2.4% on Ulmaceae, 0.5% on Rosaceae, 0.2% on Aceraceae. The infected trees all occurred between altitudes of 420 and 1,250m above sea level, but most were between 600 and 900m. *L. tanakae* was not seen at altitudes below 420m. The mean DBH and heights of parasitism were 38.6cm and 13.0m. The number of *L. tanakae* per host tree ranged from 1 to 22 (mean 3.0) with a mean of 2.9 *V. album* var. *coloratum* per host. *L. tanakae* and *V. album* var. *coloratum* are competing with each other. Twig death was also observed on 300 host trees (71.43%) and 2.1 twig per tree. Most of all *L. tanakae*'s population and habitat have been fragmented, therefore regular monitoring has to be carried out for conservation of *L. tanakae*.

HOST SPECIES INVENTORY OF *VISCUM ALBUM* VAR. *COLORATUM* (KOREAN MISTLETOE) IN CENTRAL DISTRICT OF SOUTH KOREA

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Evergreen, stem-parasitic Korean mistletoes consist of five species and four genera in two families. In the Santalaceae, the species are *Viscum album* var. *coloratum* and *V. album* for. *Rubroaurantiacum*, and in the Loranthaceae, *Pseudixus japonicus*, *Loranthus tanakae*, *L. yadoriki*. *V. album* var. *coloratum* is widespread throughout Korea as evergreen. Several studies on the Korean mistletoes have been conducted in terms of their population, distribution and host plants but are insufficient in comparison to the European mistletoes, which have been extensively researched.

This paper, therefore, represents the first attempt to evaluate the distribution of mistletoes and to identify host species of *V. album* var. *coloratum* in Korea. Mistletoes and their host species were examined in an extensive field survey in Gyeong-gi and Gang-won province in the middle of Korean Peninsula. Twenty sites at altitudes of 0 ~ 1,200 m were studied in a transect 20 m wide and 2 km long (4 ha). Host trees parasitized by *V. album* var. *coloratum* comprised 22 species belonging to 14 genera, and 8 families (Aceraceae, Betulaceae, Ebenaceae, Fagaceae, Rosaceae, Salicaceae, and Ulmaceae). The Fagaceae were parasitized by to the extent of more than about 85%. Generally, mistletoe prefers the edge of forests or the topside of trees, however, this research indicated that Korean mistletoe appeared both on the topside and in the middle of host plants, so it was ascertained that Korean mistletoe was less affected by the environment. It was also found that all the recorded host plants of *V. album* var. *coloratum* as hyper-parasitism were eudicotyledons without the gymnosperms and herbaceous species.

FLORAL BIOLOGY OF *HYDNORA ABYSSINICA* – NEW INSIGHTS FROM SOUTHERN NAMIBIA

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Our research on the holoparasitic genus *Hydnora* in Southern Africa spanning almost two decades, has concentrated on the *Euphorbia* parasitizing clade with *H. visseri*, *H. africana, H. longicollis* and *H. triceps*. *H. abyssinica*, one of the Fabaceae parasitizing taxa, remained the "forgotten species". Our observations indicate that *H. abyssinica* may remain vegetative for extended periods of time and has a very short flowering period in response to rain. This may explain the difficulty to locate populations despite the fact that it is regarded as the most widespread *Hydnora* species. A new focus on this species during the past two years documents the distribution of the species in Namibia, with a number of populations being identified which are large enough to carry out studies of their floral biology. Floral metrics revealed large variations in osmophore dimensions - the biological significance of which will be investigated in more detail in the near future. Floral length (and thus the depth of the androecial and gynoecial chambers) varied between 10 and 38cm. As there is little variation in the above soil length of flowers (12.7cm ± 3.32), this is attributed to differences in the depth of the rhizome. We present new insights into the floral biology of this species parasitizing *Acacia karroo* in the Gondwana Cañon Park in southern Namibia. Preliminary data on insect visitation indicates the importance of scarab beetles rather than the hide beetles as being responsible for pollination in *H. visseri* co-inhabiting the same area. The rather flexible antheral ring of *H. abyssinica* allows the entrance of beetles slightly larger than the diameter of the antheral opening (9.92mm ± 0.89). Bud opening seems to be triggered by rain and is synchronized with a sudden increase in the abundance of insects. Herbivory in this species is much lower than what was found for *H. visseri* in the same area - this may be attributed to the protection provided by dead *Acacia* branches typically covering the *Hydnora* buds, flowers and developing fruits. The ability of *H. abyssinica* to kill its host was also demonstrated.

SANTALALES PHYLOGENY PROMPTS NEW INSIGHTS INTO MORPHOLOGICAL CHARACTER EVOLUTION

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Among flowering plant parasites, Santalales is the largest order with 18 families, 160 genera and approximately 2280 species. Individual molecular phylogenetic analyses have been conducted for all families but until now all available taxa and genes have not been analyzed simultaneously. Maximum likelihood analyses of 146 Santalalean genera using a suite of seven nuclear and chloroplast genes were conducted. These allowed branch lengths to be calculated based on specific molecular evolutionary models. In addition, a morphological character matrix was assembled for these genera and this was linked to the molecular tree using the software package, Mesquite. Ancestral state reconstructions were then made to study character evolution on the phylogenetic tree. As frequently seen in these plants and other organisms, the pattern of morphological character variation often does not correspond to the molecular tree topology (i.e. homoplasy in the context of parsimony). Examples of characters showing "parallelism" or "convergence" include aerial parasitism, plant sexual condition, ovary position, loss of ovular integuments, and attachment structures on seeds. The presence and absence of such character states in the data matrix may, in some cases, reflect the presence or absence of the genes underlying the morphological trait. It is proposed here that more often such "homoplasy" is better described as atavism (commonly referred to as a "throwback", i.e. reappearance of a trait after being evolved in the ancestor and lost in descendants). For homologous genes, which cannot be lost and evolve again; it is more likely that their pattern of expression changes over time throughout the phylogeny. It is argued that evolution-development approaches combined with genomics will provide powerful tools to further explore this phenomenon in Santalales and other parasitic angiosperms.

SUGAR METABOLISM DURING GERMINATION OF *OROBANCHE MINOR* AS A NOVEL TARGET FOR SELECTIVE CONTROL

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Parasitic weeds, *Striga* spp. and *Orobanche* spp. cause serious damage to agriculture worldwide. A novel and effective strategy for 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 parasitic-specific biological events is important for developing selective control strategies. We focused on the germination process of parasitic weeds to find biological events specific to these species and conducted metabolic profiling of *Orobanche minor* seeds. Consequently, it was found that gentianose, a rare trisaccharide, consisting of a fructose and two glucose elements, decreased concurrently with increases of glucose and fructose, immediately after treatment with the germination stimulant, GR24. This result indicated that the glucose and/or fructose, which provide the energy required for seed germination, are supplied by the hydrolysis of gentianose. A potent inhibitor of glycosyl hydrolases, nojirimycin bisulfite (NJ), decreased not only the germination percentage of *O. minor* but also that of *Striga gesnerioides*. From these results, it is hypothesized that gentianose metabolism is crucial for germination of these parasitic weeds and the key enzyme in the gentianose metabolic pathway could be a novel target for selective control of parasitic weeds. To characterize the enzymes involved in gentianose metabolism, we extracted crude enzyme from *O. minor* seeds and measured glycosyl hydrolyzing activity. As a result, it was shown that the trisaccharide gentianose was hydrolyzed to monosaccharides via the disaccharide gentiobiose. The gentiobiose-hydrolyzing activity in the cell wall/membrane binding protein fraction was increased during the germination process and reached its maximum four days after GR24 treatment. Additionally, the gentiobiose-hydrolyzing enzyme was inhibited selectively by NJ. Among the related sugars, only glucose could restore the germination percentage when they were applied with NJ, indicating glucose produced from gentiobiose is crucial for the germination process of the parasitic weeds. Some other inhibitors of glycosyl hydrolases also inhibited the germination of *O. minor*. It is concluded that the gentiobiose-hydrolyzing enzyme could be a novel target for selective control of these parasitic

DEVELOPMENT OF A HIGH-THROUGHPUT METHOD FOR ESTIMATING THE GERMINATION OF BROOMRAPE SEEDS

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The parasitic weed *Phelipanche ramosa* L. Pomel (syn. *Orobanche ramosa*) develops at the expense of many plants including wild and cultivated species. Germination of *P. ramosa* seeds is triggered by molecules exuded from the host's root. Germination percentage is usually evaluated by counting germinated seeds under a binocular microscope. This method is time-consuming, tedious due to the minute size of broomrape seeds (about 200 µm in diameter), and inappropriate if hundred of samples have to be analyzed. To make easier and faster measurements within high-throughput studies, a standardized multiwell plate germination test was developed coupled with spectrophotometric reading of methylthiazolyldiphenyl-tetrazolium bromide (MTT) reduction. Surface-sterilized broomrape seeds were suspended in sterile water (10 g L^{-1}) and distributed in multiwell plate (50 µL ≈100 seeds per well). After moist preconditioning (7 days, 21°C, in a dark, sealed plate), the synthetic germination stimulant GR24 was added at different concentrations to the wells, and volumes were adjusted to 100 µL with sterile water. Controls were made with solvents and without seeds. Plates were incubated for germination in a dark, sealed plate, at 21°C. After various durations of incubation, from two hours to seven days, ten µL of MTT solution (5 mg mL^{-1}) were added per well and plates were returned to incubator overnight. Then, 200 µL of MTT solubilization solution (10% Triton X-100, 0.04 M HCl in isopropanol) was added per well and plates returned to incubator for a further 5 hours. The background absorbance was then measured at 630 nm and subtracted from the absorbance at 570 nm. Germ tubes came through seed coats on the third day in the presence of GR24. MTT reduction by dehydrogenase activities from germinating seeds was optimal at this time. Then activities were located specifically in t he seed body and at the germ tube apex. Over three days in the presence of GR24, germ tubes elongated and MTT reduction was constant and optimal. As the relationship between reduction of MTT and germination percentage on the third germination day under various GR24 concentration was linear, absorbance could be used to estimate germination percentage. Several applications are proposed for this high-throughput germination test, such as molecular screening for determination of the dose-response of natural and xenobiotic molecules, or accession screening for breeding resistance considering the activity of root exudates on *P. ramosa* germination. An expected application could be also dedicated to unravel parasite seed germination. For example, the test showed that exogenous ABA inhibits germination in the presence of GR24 following a dose dependant manner.

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MORPHOLOGICAL RESPONSE OF TOMATO (*LYCOPERSICON ESCULENTUM* MILL.) TO THE PARASITIC WEED – *PHELIPANCHE RAMOSA* L. POMEL AND THE PATHOGEN – *OIDIUM NEOLYCOPERSICI* L. KISS

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Investigations were carried out in the Research Institute of Vegetable Crops (Research Institute of Horticulture now) for about 10 years on tomato cultivars Corindo F1 and Remis F1. The aim of the experiment reported here was to observe *Phelipanche ramosa* morphology and its structural connection to tomato roots. Moreover, influence of the parasitic plant on the growth and yield of tomato was investigated.*P. ramosa* has a wide host range and cause severe yield losses to numerous important cultivated plants. In the experiment tomato seeds were sown in multipots in the glasshouse and then transplanted to the bigger pots, selecting seedlings with about 1g of branched broomrape seeds. Bringing these two plants together in a controlled system gives the possibility to observe development of plants and interactions between them. Fruits were harvested every 3-7 days from July to November. *P. ramosa* fragments with tomato roots were collected to anatomical study. Preparations for microscopical examination were made using a paraffin method. Tissues were then analyzed with Nikon Eclipse 80i and Olympus SZX16 microscopes. *P. ramosa* stems are yellow-brown with violet flowers. Every flower has four anthers in which a very large number of pollen grains is produced. Analysis of stems revealed that the epidermis formed multicellular, secretory hairs. Many starch grains were observed in the parenchyma cells in the highest part of the stem. The presence of starch indicates on the connection of parasite and host phloem elements. *P. ramosa* cells create characteristic polymorphic cells which can mediate the transport of nutrition substances from the host to the parasitic plant. Underground the parasite produces the tubercle which in contact with host roots penetrates it. The broomrape tubercle consists of a cork layer, parenchyma tissue and vascular elements with xylem and phloem in the system characteristic for stems. Vascular bundles were visible also in places, outside the central vascular cylinder. New vascular cells were formed from parenchyma cells where cell divisions were initiated following the parasite-host contact. The presence of the parasite significantly reduced the height and yield of the tomato host. Tomato plants with branched broomrape were treated with calcium nitrate to verify *P. ramosa* sensitivity to the most common fertilizer. During one of the experiments symptoms of powdery mildew were visible only on the tomato leaves with branched broomrape. In subsequent studies, authors plan to examine whether the branched broomrape could reduce the tolerance of tomato to powdery mildew.

EMBOLISM FORMATION IN THE WOOD OF *TAPIRIRA GUIANENSIS* (ANACARDIACEAE) BY THE PRESENCE OF THE HEMIPARASITE *PHORADENDRON* (SANTALACEAE)

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The genus *Phoradendron* – the most diverse in the Santalaceae family - comprises about 200 species that infest a wide range of trees, being more diversified in the tropics. In Brazil, a frequent host is *Tapirira guianensis*, a tree which is widespread in the country especially in gallery forests. It presents indistinct or absent growth rings, diffuse porosity and no definite distribution pattern of vessels, ranging from solitary to multiples of 4 and low density wood, which is used for making toys and home furniture. Another frequent use includes reforestation of degraded areas, and its fruits are highly consumed by wildlife in general. In view of these uses, studies evaluating the possible impact of parasite in the wood are needed. This study investigated the wood anatomy of 21 samples of varying diameters of *T. guianensis*, of which eleven were parasitized and ten healthy. Parasitized and non-parasitized branches with host leaves were removed from the trees and quickly submerged in filtered water, when they were sectioned again about 30 cm from the extremity and the vessel openings were surfaced and de-obstructed with sharp blades. All branches were then carefully transferred to Safranin (0,1% - 0,01%) solutions, to avoid the introduction of new embolisms. After 3 to 12 hours the branches were removed from the solution and air-dried. Parasitized branches were cut into portions upstream and downstream from the parasite's point of infestation, thus creating two groups (upstream and downstream). The anatomical features of the wood evaluated were tangential vessel diameter and vessel frequency, for both functional (stained) and non-functional (unstained) vessels. The statistical analysis showed a small variation in vessel diameter between the non-parasitized (narrower vessels) and the parasitized (wider vessels) branches. The non-parasitized ones showed about 27% more functional vessels than the parasitized group, especially when comparing upstream versus downstream portions, with less than a third of the vessels being functional above the location of the parasite infection . Therefore we conclude that the infestation by parasites of the genus *Phoradendron* increases the frequency of non-functional vessels, especially at the downstream portion of the parasite's infection point and also slightly influences the growth of new vessels.

MICROMORPHOLOGICAL STUDIES ON SEED OF *OROBANCHE* L. (OROBANCHACEAE) SPECIES FROM TURKEY, AND THEIR SYSTEMATIC SIGNIFICANCE

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The genus *Orobanche* occurs as holoparasitic on a wide range of vegetable crops and legume pulses. A comprehensive morphological study was carried out on the seeds of 114 taxa from *Orobanche*. The use of seed surface characters, from gross morphology to subcellular level, is of great importance, particularly in *Orobanche*, which possesses a much reduced vegetative growth and consequently provides few vegetative characters for taxonomic purposes. The present study is concentrated mainly on the outer seed wall ornamentation. A dichotomous identification key will be presented along with seed descriptions, measurements and morphological details, LM photos and SEM micrographs. In addition, phenetic analysis of seed characters revealed that several groups of species in *Orobanche* appear to be closely related on the basis of their seed features. The relationships of species groups will be discussed. Our results indicate that characters such as size, shape and color of the seeds are not very useful in differentiation of the taxa. However, other characters of the epidermal seed coat cells proved to be very helpful in this respect. Ornamentation of the periclinal walls could be used to discriminate five morphological types. Seed coat sculpturing in the genus *Orobanche* sect. *Orobanche* and sect. *Trionychon* is discussed in relation to taxonomy. Based on outer seed wall ornamentation, five different types are recognized: fibrillar, papillate, smooth, perforate and granulate-rugulose. Types I and II correspond to the section *Trionychon*, whereas types III, IV and V are included in the section *Orobanche*. These results therefore support the division of the genus into two sections, *Trionychon* and *Orobanche*.

Symposium

ADVANCES IN STRIGOLACTONE RESEARCH

Chair

Joseph HERSHENHORN and Hinanit KOLTAI

STRIGOLACTONE SUBSTANCES STIMULATE DIFFERENT GENE EXPRESSION OF TOMATO LIGHT HARVESTING COMPLEXES AND HYPHAL GROWTH OF ARBUSCULAR MYCORRHIZAL FUNGI

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Strigolactones (SLs), previously characterized as seed germination stimulants of the parasitic plants *Striga* and *Orobanche*, were also characterized as active molecules that influence AMF spore germination, hyphal branching and growth at very low concentrations. In addition, SLs were found to induce the formation of light harvesting complexes (LHC) in the plant: Recently, we have found that synthetic SL molecule, GR24, has a marked increase in the transcriptional level of several tomato genes that are associated with LHC in the chloroplast. Also, the lower level of chlorophyll found in a tomato mutant that does not produce SLs, imply that SLs may affect plant physiology by inducing light harvesting. In the present study the ability of different SLs chemical analogs to induce LHC and mycorrhiza hyphal branching was examined. While GR24 was able to induce both the hyphal brunching and LHC genes expression, some of the tested analogs were able to induce hyphal branching but not the LHC genes expression. These results suggest that diverse molecules may affect differently plant physiology or plant-mycorrhiza communication; this notion will be presented and discussed in details.

THE SYNTHETIC STRIGOLACTONE GR24 INFLUENCES THE GROWTH PATTERN OF PHYTOPATHOGENIC FUNGI

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Strigolactone that are released by plant roots are involved in both plant symbiosis with arbuscular mycorrhizal fungi and in plant infection by root parasitic plants. We examined the response of phytopathogenic fungi to the synthetic strigolactone GR24. Imbedded in the growth medium, GR24 inhibited growth of the root pathogens *Fusarium oxysporum* f. sp. *melonis*, *Fusarium solani* f. sp. *mango*, *Sclerotinia sclerotiorum* and *Macrophomina phaseolina*, and of the foliar pathogens *Alternaria alternata*, *Colletotrichum acutatum* and *Botrytis cinerea*. In the presence of this synthetic strigolactone, intense branching activity was exhibited by *S. sclerotiorum*, *C. acutatum* and *F. mango* and *B. cinerea*, whereas suppression of hyphal branching by GR24 was observed in *M. phaseolina*. These results suggest that strigolactones not only affect mycorrhizal fungi and parasitic plants: they also have a more general effect on phytopathogenic fungi.

STRIGOLACTONES' MULTIPLE ROLES IN PLANT DEVELOPMENT

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Strigolactones (SLs) were defined as a new group of plant hormones. They were shown to suppress lateral shoot branching, and thereby to regulate shoot architecture. SLs were also shown to alter root architecture and to affect root-hair elongation, suggesting a biological role for SLs in root development, in addition to that in the shoot. Also, SLs were shown to be involved in plant seed germination. The effect of SLs on plant development is conducted as part of a network of plant hormones that coordinate root and shoot development: cytokinin, auxin and ethylene were suggested to be involved in SLs' effect on plant development. Moreover, on the one hand, growth conditions were shown to influence the SL regulation on plant growth, and on the other, SLs synthesis was shown to be growth-conditions dependent. These findings suggest that SLs play a biological role in plants as coordinators of shoot and root development in response to nutritional and light conditions. However, SLs are also signal molecules that facilitate parasitic plants seed germination and hyphal branching of the symbiotic arbuscular mycorrhizal fungi. The roles played by SLs in plant development and communication may imply on their evolution. SLs were likely to evolve as modulators of plant development or as enhancers of beneficial plant symbiosis, and may have been exploited by the parasitic plants.

SCREENING AND IDENTIFICATION OF MAX2-INTERACTING FACTORS FOR THE ISOLATION OF NOVEL STRIGOLACTONE-SIGNALING FACTORS

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Strigolactones (SLs) have been known as germination stimulants of root parasitic plants such as witchweeds (*Striga* spp.) and broomrapes (*Orobanche* and *Phelipanche* spp.), both of which cause devastating damage to important crops in many part of the world. SL is also known as a hyphal branching factor of symbiotic arbuscular mycorrhizal fungi and as a hormone that regulates shoot branching. Although inhibition of SL-induced seed germination of parasitic plant is a critical issue, there is no information about SL-recognition and SL-signaling in parasitic plants. To obtain clues about SL-signaling mechanisms in parasitic plants, we aimed to reveal SL-signaling mechanisms in non-parasitic plants, such as rice and Arabidopsis. AtMAX2 is an Arabidopsis F-box protein proposed as an SL-signaling factor, although its function is largely unknown. To identify novel SL-signaling factors that function with AtMAX2, we performed yeast two-hybrid (Y2H) screening by using AtMAX2 as a bait. Finally, we obtained seven AtMAX2-interacting factors and three of them belonged to the NF-YC transcription factor family. We are conducting further experiments using knockout and overexpression mutants of these genes to investigate whether AtMAX2 and NF-YCs are involved in the SL-signaling mechanism in Arabidopsis. Besides, it is reported that gibberellins (GAs) affect shoot branching in rice. Thus we investigated whether there exists a cross-talk between GA-signaling and SL-signaling. We found that some GA-signaling factors interacted with AtMAX2. On the basis of these data, we suggest that AtMAX2 functions with multiple factors in SL-signaling and in cross-talk between SL-signaling and GA-signaling.

NEW POTENT FLUORESCENT ANALOGUES OF STRIGOLACTONES: SYNTHESIS AND BIOLOGICAL ACTIVITY IN PARASITIC WEED GERMINATION AND HYPHAL BRANCHING IN AM FUNGI

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Several hundreds of different organic compounds released from plants, insects, animal and microbes are known to affect the growth, development and distribution of the receiving organism. Among them are signalling molecules, which play a key role in the intricate communication network in the soil. In this context, a new class of signalling molecules, the strigolactones (SLs), has deserved a particular interest both by biologists and chemists. SLs were originally isolated from plants roots exudates as germination stimulants for root parasitic plants of the family Orobanchaceae, including witchweeds (*Striga* spp.), boomrapes (*Orobanche* and *Phelipanche* spp.) and *Alectra* spp. and so were regarded as detrimental to the producing plants. Subsequently, their role was unveiled as indispensable chemical signals for the establishment of arbuscular mycorrhizas (AM), the widespread symbiosis between most land plants and a small group of soil fungi. In addition to these functions in the rhizosphere, it has recently been shown that SLs represent a new class of plant hormones that inhibit shoot branching. The concentration of these stimulants in the roots exudates is extremely low, which hampers their isolation and characterization as well as their use in agriculture. Extensive studies on structure-activity relationship have been conducted both for natural and for synthetic SLs analogues. Very recently Akiyama *et al.* demonstrated that the structural requirements in AM fungi are very similar but not identical to those observed in root parasitic weeds, especially with respect to the enol ether bridge in the C-D part. Increasing evidence suggests that the induction of seed germination in parasitic weeds or hyphal branching in AM fungi proceeds *via* a receptor-mediated mechanism. So far very little is known about the protein structure of this hypothesized receptor nor its location. In this sense labelled synthetic active strigolactones can be considered as useful tools for the detection of the receptor *in vivo* or for protein fishing experiments. We recently reported the synthesis of a new class of SL analogues featuring an unprecedented extended conjugate system and whose bioactiphore is an ,-unsaturated ketone instead of the more common ,-unsaturated lactone. All these new molecules showed a remarkable activity on *P. aegyptiaca* seeds germination tests. Furthermore, some of these molecules showed interesting luminescent properties that prompted us to the design and the development of new fluorescent synthetic analogues whose spectroscopic properties could be exploited in bioimaging studies. In this work we report the synthesis of new fluorescent analogues of Strigolactones, their spectroscopic properties and the evaluation of the biological activity both on seeds of *P. aegyptiaca* and on the AM fungus *Gigaspora margarita*.

STRIGOLACTONES: A CRY FOR HELP RESULTS IN FATAL ATTRACTION. IS ANY ESCAPE POSSIBLE?

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During evolution, plants have adapted an ecological balance with their associates, competitors, predators and pests. Keeping this balance intact is an active process during which the plant needs to respond to many different stimuli in order to survive. For example, plants have developed an array of physiological and biochemical responses to phosphate deprivation. One of these responses is the induction of strigolactone biosynthesis.

Strigolactones that are exuded from the plants' root into the rhizosphere stimulate the formation of symbiotic associations of plant roots with arbuscular mycorrhizal (AM) fungi. AM fungi colonize the root cortex to obtain carbon from their host while assisting the plant in phosphate acquisition. Increased strigolactone production as a result of low phosphate availability is therefore considered as a cry for help for mycorrhiza, and was believed to explain its persistence during evolution despite the fact that strigolactones also stimulate the germination of parasitic plant seeds. The discovery that strigolactones (or their metabolites) are a novel class of plant hormones involved in the inhibition of shoot branching has shed new light on these evolutionary aspects. An increase in strigolactone production was indeed found to be responsible for the reduction in the number of shoot branches during limiting phosphate conditions in the non-mycorrhizal plant Arabidopsis. Moreover, orobanchol was detected in xylem sap and its concentration was found to be increased under phosphate deficiency, which is consistent with the idea that root-derived strigolactones are transported to the shoot, where they regulate bud outgrowth. Because the shoot to root ratio is known to be coordinately controlled, we also investigated the contribution of strigolactones to root developmental processes. Application of the synthetic strigolactone analog GR24 had an effect on primary root length and lateral root initiation and development. Moreover, GR24 treatment resulted in decreased auxin levels in young rosette leaves. This explained the fact that the magnitude of the effect of GR24 application on root architecture was found to be dependent on the auxin status of the plant. Interestingly, under phosphate-limiting conditions, endogenous strigolactones present in wild-type plants stimulated a more rapid outgrowth of lateral root primordia when compared with strigolactone-deficient mutants.

Finally, the discovery that strigolactones also have an important hormonal function inside the plant has not only shed new light on evolutionary aspects of strigolactone production, but also has consequences for the development and application of control strategies to reduce parasitic plant infection.

INTERACTION BETWEEN STRIGOLACTONE AND OTHER PLANT HORMONES

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Seeds of root parasitic weeds need chemical stimulants released from host roots for germination. Strigolactones (SLs) are representative germination stimulants that *in planta* function as a novel class of plant hormones inhibiting shoot branching. Two classical plant hormones, cytokinin (CK) and auxin, are known to affect shoot branching. Therefore in the present study, effects of CK and auxin on SL production and exudation were examined.

We already demonstrated that phosphate (P) deficiency increased SL production and exudation. All CKs including *t*-zeatin, benzyladenine, kinetin and phenylurea type CPPU significantly decreased root contents and root exudation of SLs in rice plants grown under P deficiency. SL production and exudation decreased with the increase of zeatin- concentrations in culture media. Zeatin also reduced SL root levels and root exudation in SL insensitive mutant d3. Furthermore, P fertilization systemically regulated SL exudation but CK did only locally.

Exogenous application of auxin transporter inhibitors, NPA (naphtalam) and TIBA (2,3,5-triiodobenzoic acid) to hydroponic culture media suppressed root contents and root exudation of SLs in sorghum plants, suggesting that polar auxin stream is necessary for SL production in roots. Accordingly, application of CK or anti-auxin would reduce damages caused by root parasites by reducing SL exudation.

Session 3

ECOLOGY AND POPULATION BIOLOGY

Chair

Yaakov GOLDWASSER and Garifalia ECONOMOU

STATUS OF *STRIGA HERMONTHICA* (Del.) BENTH IN SMALL-SCALE FARMS IN BENUE STATE, NIGERIA

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Extensive and intensive surveys were carried out in the year 2010 in the three agricultural zones of Benue State to determine the extent or status of *Striga hermonthica* (Del.) Benth infestation on maize in low in-put farms. For the extensive surveys, structured questions in form of questionnaire were the instrument used to obtain information from farmers. These were administered through the Extension Staff of the Benue State Agricultural and Rural Development Authority (BNARDA). An intensive survey was conducted on a maize/cassava intercrop farm mainly infested with *S. hermonthica* in Waapera village, Ushongo Local Government Area in Benue State, Nigeria. The results of the surveys indicated a widespread occurrence of *S. hermonthica* in all the agricultural zones of Benue State, with the Northern zone having the highest level of infestation (62%) followed by Eastern (46%) and then central (16%) zones. On the farm where an intensive survey was carried out, there was total crop failure (100% grain yield loss) due to *S. hermonthica* parasitism on maize. It was concluded that *Striga* is a serious weed and a major constraint to maize production in Benue State. Also, that the management practices employed by farmers in the State are not helping the problem of *Striga* infestation. This is the major reason why farmers can not cope with *Striga* parasitism and many of them have stopped growing maize. It is recommended that an integrated management strategy for *Striga* control be employed by farmers to help reduce *Striga* problem in small holder farms.

SUNFLOWER PRODUCTION AND THE BROOMRAPE PROBLEM IN CHINA- PAST AND PRESENT SITUATION

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Eighteen provinces grow sunflower in China, but it is mainly grown in northern and northwestern China. Heilongjiang province cultivates the largest area of sunflower, followed by Jilin, Inner Mongolia, Liaoning provinces and Xinjiang Uygur Autonomous Region. The highest yields occurred in Inner Mongolia in 1997, and in Jilin in 2004. Both Inner Mongolia and Jilin displayed a high fluctuation in the yield. The other provinces yields also change each year, but there is not much variation. *Orobanche cumana* infestation of a sunflower field was first recorded in Zhaoxian county of Heilongjiang province in 1959. In the early 80's of the 20th century it was recorded in all of the sunflower production areas in the Heilongjiang province, but it was not severe at that time. Most of the sunflowers were planted in barren salinized soil in Heilongjiang province by continuous cropping, which resulted in the *Orobanche* problem. In 1979, *O. cernua* was recorded in Changling county of Jilin province. In all counties of Baicheng city of Jinlin province both *O. cernua* and *O. coerulescens* occurred in sunflower fields. In Huailai county of Hebei province it was reported that in 1991, 3000 to 4000 ha of sunflower fields were infested by *O. cumana*, which was 80% of the total sunflower planted and the yield reduction was 30-50%. It was reported in 1988 that in Bole city of Xinjiang 80% of sunflower plants were parasitized by *O. cumana*. In Shilou county of Shanxi Province *O. cumana* was discovered in 2003 on sunflower. In Dingbian county of Shaanxi Province crop loss due to broomrape was up to 48% in 2002. Sunflower production in China ranks number three in the world and the broomrape problem occurs in all sunflower production areas.

GENETIC DIVERSITY OF *STRIGA HERMONTHICA* POPULATIONS IN ETHIOPIA: EVALUATING THE ROLE OF GEOGRAPHY AND HOST SPECIFICITY IN SHAPING POPULATION STRUCTURE

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Striga hermonthica, a root hemiparasitic Orobanchaceae, severely constrains grain production in sub-Sahara Africa. Host specificity and geography may play important roles in shaping the population structure of *S. hermonthica*, with the Rift Valley potentially presenting a significant barrier to dispersal. Genetic diversity was assessed in 12 *S. hermonthica* populations from locations in Ethiopia. Of these, seven populations were parasitic on sorghum, two each on tef and maize, and one on finger millet. Genetic variation was detected using four AFLP primer combinations. After correcting for repeatability, 385 fragments were detected across all primer combinations. The percentage of polymorphic loci was relatively high, ranging from 53.2% to 76.4%. Expected heterozygosity ranged from 0.168-0.279. Genetic differentiation between populations was relatively high and all populations were significantly different from each other. FST values ranged from 0.032 to 0.293 and averaged 0.146. Genetic differences between populations could not be attributed to host specificity. Instead, geography was the main determinant of population structure. There was a correlation between geographic and genetic distance. A significant portion of the genetic variance could be apportioned on either side of the Rift Valley (5%; p=0.001). Also, a significant geographic barrier was identified in the southern portion of the sampled region.

THE GENUS *HYDNORA* (HYDNORACEAE) IN SOUTHERN AFRICA AND MADAGASCAR

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During the past twenty years we have investigated the genus *Hydnora* in southern Africa (Botswana, Zimbabwe, South Africa, Namibia) and Madagascar. The results of those researches are summarized here. There are seven species in two sections of the genus *Hydnora*. Subgenus *Hydnora* Thunb. contains *H. africana* Thunb., *H. longicollis* Welw., and *H. triceps* Drége & E. Mey. along with the recently described *H. visseri* J. Bolin, E. Maass and L. J. Musselman. *Hydnora triceps* is unique among Eudicots in flowering underground. We have named *H. visseri* in honor of the pioneer researcher on parasitic plants in southern Africa, the late Johann H. Visser. Members of the subgenus *Hydnora* are characterized by angled rhizomes, pink to orange-red interior perianths, recessed osmophores producing fetid odors of rotting flesh, and restriction to fleshy species of *Euphorbia* as hosts. All four species are known only from karoo vegetation of Angola, South Africa, and Botswana. The subgenus, *Dorhyna Decaisne* (including subgenus *Tricephalohydum* Harms) includes *H. abyssinica* A. Braun (= *Hydnora johannis* Becc., *H. solmsiana* Dinter), *H. esculenta* Jum. and H. Perrier, and the East African *H. sinandevu* Beentje & Q. Luke which is excluded from this study. Members of *Dodrhyna* have terete rhizomes, osmophores as a distinct cucullus on the tip of the perianth lobe, and are restricted to hosts in the Fabaceae. *Hydnora johannis* is the most widespread member of the family Hydnoraceae, extending from Namibia to Oman and Saudi Arabia. Further work is needed to determine the taxonomic status of some its segregates. *Hydnora esculenta* was originally described as being dioecious. Our work in Madagascar shows that, like all members of the genus, this species has perfect flowers, the confusion arising over incomplete type material. The floral biology of trap flowers, host selection, and phylogeny are discussed.

PARASITIC PLANTS IN CYPRUS FLORA

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Cyprus, a geologically and biogeographically isolated Mediterranean Island, has high diverse flora. In all island 1500 plants species have been recorded and 1300 species in Northern Cyprus of which 143 are endemic. In recent years, 85 alien plant taxa have been introduced to the island and have established as adventives mainly in cultivated areas. Fourteen parasitic plant species have been recorded from four families which are Rufflesiaceseae, Orobanchaceae, Cuscutaceae, and Santalaceae. Orobanchaceae has the highest number of species: *Cistanche phelypaea* (L.) P., *Phelipanche aegyptiaca* Pers., *P. ramosa* L., *Orobanche orientalis* Beck., *O. cypria* Reuter, *O. alba* Stephan ex Willd., *O. crenata* Forsk, and *O. minor* Sm. *O. minor* and *P. ramosa* have lower taxons too. Most of Orobanchaceae species are weeds in crops. *P. ramosa* and *P. aegyptiaca* infest tomato greenhouses in Northern Cyprus although they are not heavily infested due to soil solarization. Faba bean fields in the Kyrnea and Guzelyurt areas are infested by *O. crenata*. Three *Cuscuta* species, *C. campestris* Yunck., *C. palaestina* Boiss., and *C. planiflora* Ten., have been recorded on herbs. During our investigations in the last two years, we have noticed that *C. campestris* infested larger areas than it had been recorded previously. It is found in Famagusta and Nicosia it was recorded in Rizokarposa in the literature. *C. monogyna* is reported on vitis and fruit trees such as pistachio and citrus. *Thesium humile* Vahl., *Osyris alba* L., and *Cytinus hypocistis* L. are the other parasitic plant species recorded in the flora of Cyprus. Only *O. cypria* is an endemic parasitic plant species. Most of the records of parasitic plants in Cyprus are older than 100 years and thus need to be updated.

GENETIC DIVERSITY OF WILD *OROBANCHE CERNUA* L. POPULATIONS FROM SOUTHEASTERN SPAIN

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Orobanche cernua L. is an autochthonous species in Spain. It is mainly distributed in the Mediterranean basin and in markedly arid areas eastwards. *Orobanche cernua* was described for the first time in Spain on materials collected near Aranjuez, central Spain in 1758. It parasitizes different species of the Compositae, being most frequently found on plants of the genus *Artemisia*. The objective of this analysis was to study the genetic diversity among 11 populations of *O. cernua* collected on wild hosts of *Artemisia* spp. (*A. barrelieri* Besser and *A. campestris* L. subsp. *glutinosa* [J. Gay ex DC.] Batt.) and *Launaea lanifera* Pau in the south-east area of Spain (Jaén, Granada and Almería provinces). The analysis of DNA samples (pools of 1-5 broomrapes, depending on availability) from these populations with 47 RAPD primers identified a total of 776 intense fragments, after excluding monomorphic bands of the original database. The size of the scored amplified fragments was found in the range of DNA ladder between 2,652 and 300 bp. The number of bands per primer ranged from 2 to 32 with an average of 16.5 fragments per primer. Cluster analysis using the UPGMA method showed a good fit to the matrix on which it was based. Mantel test using 1000 permutations revealed a highly significant cophenetic correlation coefficient ($r = 0.99$, $P < 0.0001$). Principal coordinates analysis on the similarity matrix showed that the first three eigenvalues explained a percentage of the total variability of 58.0, 15.2 and 9.1%, respectively. The dendrogram obtained by the UPGMA method based on Dice index matrix showed a clear separation between samples of *O. cernua* in terms of geographic origin. Populations of *O. cernua* were clustered into five groups. A first group consisted of three subgroups that included the native populations of Almería (into two subgroups) and the populations from Jaén. A more distanced group included the populations collected in Granada and one population from Almería genetically more distant to the previous ones. Comparative studies of genetic diversity among species of the genus *Orobanche* have been carried out by analyzing the genetic behavior of *O. cernua* populations with other populations of *Orobanche* spp. This study analyzes the genetic variability of a set of *O. cernua* populations between them and not in relation to other *Orobanche* species. The results of the analysis showed a high genetic distance among *O. cernua* populations, indicating the existence of high genetic diversity for this species in Southeastern Spain.

DISTRIBUTION OF *RAFFLESIA* POPULATIONS IN UPPER PERAK, PENINSULAR MALAYSIA

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Rafflesia is a spectacular flower that never fails to impress anyone who encounters it in the forest. Four species occur in Peninsular Malaysia. Although *Rafflesia* is rare and vulnerable, populations of three species, *Rafflesia azlanii*, *R. cantleyi* and *R. kerrii*, are all found in forests at Upper Perak. Research was carried out in the Gerik District, Upper Perak which includes the Temenggor Forest Reserve (FR), the Royal Belum State Park and Bersia to map *Rafflesia* distribution, to identify the species of *Rafflesia*, and to study their population status. The Royal Belum State Park is important to conserve populations of *R. cantleyi* and *R. kerrii* and the Temenggor FR is home to *R. azlanii*. These are the only accessible protected areas for the three species in Peninsular Malaysia. Most populations are found near the river draining from Temenggor Lake, but a few populations are also found in forest around Gerik. Other easily accessible populations of *R. azlanii* and *R. cantleyi* are in the Bersia area. The proposed North-West Peninsular Malaysia forest area will be an important element in the conservation of *Rafflesia* in Peninsular Malaysia and will compliment Taman Negara where only one species, *R. cantleyi*, occurs. In fact, the Royal Belum State Park is the only safe haven for all three species. The year-long study of distribution of *Rafflesia* populations in the Belum-Temenggor forest complex involved staff from the Royal Belum Sate Park, local people, including those involved in tourism associated with the lake and also indigenous people who were the informants of *Rafflesia* sites around Temenggor Lake. Identification of species was based on morphological features of flowers. Flowers in the Temenggor FR showed similar morphological characters to both *R. azlanii* and *R. cantleyi*. There is much diversity in lobe perigone pattern and results of the t-test confirmed the variation among the specimens. However, based on ramenta features, all specimens in Temenggor FR were identified as *R. azlanii*. In the Royal Belum State Park, there are two species of *Rafflesia*; *R. kerrii* and *R. cantleyi*. All buds were marked and their size measured and a sketch map of their distribution prepared. There are 17 *Rafflesia* localities in the Belum Temenggor area: seven in the Royal Belum State Park (Sungai Ruok, Sg. Selantan, Sg. Kejar, Sg. Tan Hain, Kg. Belum Lama and Sg. Kenarong) and ten in Temenggor FR (Pulau Besar, Kiroi, Sungai Dok, Sungai Kenyer, Chuweh, Kampung Chiong, Sungai Cap, Sg. Halong and Sg. Tebang). With all the three species occurring in the Belum-Temenggor FR, it is therefore the richest locality in *Rafflesia* species diversity in Peninsular Malaysia and therefore of critical conservation importance.

FLOWER VOLATILES OF OROBANCHACEAE- A USEFUL TOOL FOR PHYLOGENY

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Flowers produce hundreds of different volatile compounds that give them their specific odor and function to attract pollinators. The flower volatile composition is highly species-specific. This study aimed to use, for the first time, the floral volatile organic compounds (VOCs) of European broomrapes to infer the relationships between the genera *Orobanche* and *Phelipanche* with an emphasis on weedy species. The VOCs emitted from fully flowering broomrape shoots were collected using dynamic headspace sampling in the field or in the greenhouse. In addition, to assess whether the host of the broomrapes affected the VOC composition, floral VOCs of dark blue (Italian origin) and pale blue (Slovak origin) flower color morphs of *P. ramosa* were trapped when parasitizing tobacco, tomato, cabbage, rape, and *Arabidopsis*. VOC profiles were analyzed by GC-MS. The patterns and differences of VOCs between different broomrapes or *P. ramosa* growing on different hosts were analyzed using principal component analysis (PCA). PCA clustered the broomrape species in distinct groups. The clustering confirmed existing phylogenetic dogma's largely, but also showed some interesting deviations, which will be discussed. The different host species did not influence overall *P. ramosa* morphology and the color of the flowers (pale blue or dark blue) stayed the same regardless of the host. However, flower VOCs significantly differed among *P. ramosa* plants growing on different hosts. Overall, it is clear that floral scents can play a useful role as phylogeny indicators. In the light of this fact, five topics will be discussed: (1) phylogenetic patterns within broomrapes (2) phylogenetic relations between broomrapes growing on annual and perennials hosts (3) distinguishing species based on flower scents (4) possible synapomorphies of weedy species (5) possible connections of broomrape floral scent differences in relation to the host plant.

OBSERVATIONS ON PARASITIC PLANTS OF MALTA WITH REMARKS ABOUT THE TERMS ROOT, STEM AND LEAF PARASITISM

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'Observations on Parasitic Plants of Malta' is the result of an extensive research effort dealing exclusively with the wild flora of the Maltese Islands which have a total surface area of approximately 316 km2. Notwithstanding this relatively small space for life, the Maltese archipelago contains a very diverse array of plants, including a number of parasites which will be introduced in this presentation. The Broomrapes *Orobanche ramosa* L. ssp. *mutelii* (F.W. Schultz) Cout., *O. crenata* Forssk., and *O. pubescens* d`Urv., are very common but *O. lavandulacea* Reichenb., *O. hederae* Vaucher ex. Duby, and *O. densiflora* Salzm. ex Reuter, are rather infrequent. The hemiparasitic Orobanchaceae *Bellardia trixago* (L.) All. and *Parentucellia viscosa* (L.) Caruel can be found occasionally in the garigue as well as in steppic habitats. The 'Malta Fungus' *Cynomorium coccineum* L. (Cynomoriaceae) and the Santalaceae *Thesium humile* Vahl, the Lesser Bastard Toadflax, are extremely rare, in contrast to *Cuscuta campestris* Yunck. with a frequent occurrence usually along the watercourses of valleys. Plants of *C. epithymum* (L.) L. are very common, particularly in the garigue. This Clover Dodder or Thyme Dodder represents the focus of this presentation for both its host specifity in Malta and the organs of hosts penetrated by the haustoria. In general, *C. epithymum* has a wide host range, preferring Thyme species, but interestingly, in Malta there are populations penetrating almost exclusively the endemic *Euphorbia melitensis* Parl. (Euphorbiaceae), in contrast to others which penetrate almost exclusively the species *Asphodelus aestivus* Brot. (Xanthorrhoeaceae, formerly Asparagaceae) and *Urginea pancration* Nym. (Asparagaceae, formerly Hyacinthaceae). In the case of those attacking monocotyledonous hosts, all the secondary haustoria of the parasite penetrate the host leaves only. Following the terminology of root and stem parasitism in its common usage (depending on the penetrated host organ), this fact has to be described as leaf parasitism: this is totally absurd. There are two other arguments which highlight the need for a change in terminology. More than 30 years ago leaf parasitism was used to describe *Hyobanche* L. species, when the subterranean leaves of the parasite develop haustoria to penetrate host roots. Furthermore, for 35 years it has been also known that hemiparasitic Orobanchaceae (e.g. species of *Melampyrum* L. and *Rhinanthus* L.), distributed in Middle Europe, can be found penetrating almost exclusively the subterranean stems (rhizomes and culms) of their hosts instead of their roots, depending on their grassy habitats. There is therefore an urgent need for the traditional terminology of root, stem, and leaf parasitism to be updated - the reason to name a root parasite, a stem parasite or a leaf parasite should not be based on the type of penetrated organ but on the origin of the haustorium (root born, stem born or leaf born). Following these updated terms, at least for the parasitic species presented here, there is no change: hemiparasitic Orobanchaceae are still root parasites and plants of *Cuscuta* species still represent an example of stem parasitism.

Session 4

HOST-PARASITE COMMUNICATION

Chair

Radi ALY and Philippe SIMIER

INTERACTIONS AND TRANSLOCATION OF MOLECULES AND MACROMOLECULES BETWEEN HOST PLANT AND BROOMRAPE

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Parasitic weeds such as *Phelipanche aegyptiaca* are obligate holoparasites that attack roots of almost all economically-important crops in semiarid regions of the world. Translocation and uptake of molecules and macromolecules from hosts to parasitic plants has been well documented in some parasites but little is known about translocation of molecules and macromolecules from a host plant to the parasitic weed *Phelipanche*. To test for peptide movement between host and parasite we have generated transgenic tobacco plants expressing a cecropin peptide (*sarcotoxin IA*), under the control of the inducible *HMG2* promoter. Transgenic lines enhanced host resistance to the parasitic weed *Phelipanche aegyptiaca* (showed higher numbers of aborted parasitization events, reduced *Phelipanche* biomass and increased host biomass). In this study it was demonstrated that *sarcotoxin IA* translocated from transgenic tobacco plants to the attached *Phelipanche* tubercles and enhanced resistance to the host. Mannitol content in the parasite is regulated by Mannose 6-Phosphate Reductase (*M6PR*) gene, an essential process to *Phelipanche* for water and nutrient uptake from the host. In our study, we used the inverted repeat technique to silence the parasite target gene - *M6PR*. It was shown that the endogenous *M6PR* mRNA from *P. aegyptiaca* tubercles or shoots grown on transgenic tomato plants harboring the *M6PR* silencing construct was reduced by 60-80%. In this case a silencing signal was translocated from transgenic host plant to the parasite tubercles to silence the target gene *M6PR* in the parasite.

To test for protein movement between host and parasite, we used transgenic tomato and tobacco lines targeting a Green Fluorescent Protein (*GFP*) to the companion cells or to the endoplasmic reticulum (*ER*) respectively. In this study it was demonstrated that *GFP* was transferred from a transgenic tomato host companion cells to the attached *Phelipanche* tubercles or shoots through phloem connections.

In addition, we have been shown that CMV can move from infected tomato plants to parasite tubercles and shoots. Moreover, CMV RNA was shown to replicate in parasite tissues and accumulate CMV-siRNA, which may indicate a silencing transmission system in the parasitic cells.

Physical connection and transfer of molecules between *Phelipanche* and its host is an important issue for this study because the movement of molecules and macromolecules from host to parasite is a key aspect of the success of our resistance strategy.

STIMULANTS OF *PHELIPANCHE RAMOSA* GERMINATION FROM OILSEED RAPE ROOTS

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Branched broomrape (*Phelipanche ramosa* L. Pomel, syn. *Orobanche ramosa* L.) is a major parasitic weed of winter-type oilseed rape (WOSR, *Brassica napus* L.) in France. According to breeding strategies adopted for other crops, the production of WOSR lines lacking germination elicitors may be effective to obtain resistant genotypes, but these molecules are still to be characterized. To date, stimulants identified from host plants including *Arabidopsis thaliana*, another *Brassicaceae*, are mostly strigolactones (SL). Thus, SL production should be considered in WOR. However, isothiocyanates (ITC) could also be implied in *B. napus*. Indeed these compounds are produced in *B. napus* rhizosphere from the hydrolysis by myrosinase-producing microflora of released glucosinolates. For the experimental purposes, 50 seeds from two *B. napus* accessions were grown in sand during 6 weeks, under normal or sterile environment. Roots and root exudates were collected following a 7-day period of phosphate deprivation, and elicitors were extracted with ethyl acetate. Root extracts were fractionated by HPLC. Stimulating activity of collected fractions was determined in plates containing preconditioned seeds. In addition, SL and ITC were looked for by LC-MS and GC-MS, respectively, in root exudates and extracts. Whatever the plant growth conditions, SL were identified neither in the non-fractionated root extracts nor in the most active fraction, which led to 82±7% germination. By contrast, this fraction contained 2-phenylethyl-ITC, a degradation product from gluconasturtiin. Concerning root exudates, extracts from non-sterile environment induced germination, while extracts from sterile environment were active only in the presence of commercial myrosinase. Otherwise, we confirmed that some SL and ITC standards induced broomrape seed germination with EC50 in the picomolar and the nanomolar ranges, respectively. Conversely glucosinolates and other degradation products (nitriles, goitrin and indol-3-carbinol) were inactive in the concentration range tested (10^{-5} to 10^{-17} M). Thus, these findings demonstrated that *P. ramosa* seed germination was mainly triggered by ITC produced from glucosinolates in *B. napus* rhizosphere, rather than by potentially exsudated SL. Therefore, *B. napus/P. ramosa* is a complex pathosystem since parasite seed germination depends on the presence of myrosinase-producing micro-organisms in the vicinity of host roots. In addition, since all glucosinolates accumulated in WOSR roots did not lead to stable ITC, focusing on the composition of root-released glucosinolates could be a strategy to breed resistant lines. Nevertheless, *P. ramosa* seeds were also highly sensitive to SL. In addition, we showed that *Bna.CCD7* and *Bna.CCD8* genes were expressed in *B. napus* roots and stems. Attempts to detect SL in WOR roots and rhizosphere were unsuccessful to date. Therefore, improvement of sensitivity of SL detection using a Xevo TQ-S device is ongoing. Finally, as control of broomrape germination might not be complete using WOSR lines deficient in stimulant ITC production, other mechanisms of resistance have to be identified in Brassica germplasm in order to breed strong resistance by complementation with low-stimulating lines. These studies are financially supported by the agro-industry Group SOFIPROTEOL and by the Group of Economic Interest, GIE PROCOLZA.

BIOLOGICAL CONTROL OF *OROBANCHE* AND *PHELIPANCHE* SPECIES BY SUICIDAL GERMINATION STRATEGY

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Orobanche and *Phelipanche* species (the broomrapes) are root parasitic plants, some of which represent serious weed problems and cause severe yield reduction of many important crops. The numerous efforts made so far have only slightly alleviated the broomrape problems, which rather appear to increase both in intensity and in bitterness. The main obstacle for long term management of broomrape infested fields is the durable seedbank.Root parasitic weeds have developed the ability to germinate only when they are exposed to germination stimulants released from host plant roots. This synchrony is vital for parasitic weed survival because they cannot survive without nutritional support fom the host. In the absence of a suitable host, they remain dormant in the soil ensuring the persistence of the parasitic seedbank.
Several classes of plant secondary metabolites are known to induce seed germination of root parasitic weeds with the strongest activity shown by strigolactones. But also other molecules structurally distinct from strigolactones have been reported to induce seed germination of some parasitic species. Dihydrosorgoleone identified from root exudates of sorghum, stimulates germination of *Striga*. Guaianolide sesquiterpene lactones present in Asteraceae species, are active as germination stimulants especifically in *O. cumana* seeds. In this communication the isolation and chemical characterization of metabolites, belong to different class of natural compounds, from root exudates of pea (*Pisum sativum*) and common vetch (*Vicia sativa*), as well as their stimulatory activity on broomrape seed germination, will be described. Stimulatory activity of these metabolites on parasitic weed germination could be exploited in suicidal germination control strategies by synthesizing and directly applying them to the field. Also, their production in plants could be increased by breeding or by genetic transformation.

NITROGEN ABSORPTION, TRANSLOCATION AND FLUXES IN THE *PHELIPANCHE RAMOSA / BRASSICA NAPUS* INTERACTION

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The holoparasitic plant *Phelipanche ramosa* L. Pomel (syn. *Orobanche ramosa*) strictly relies on nutrient uptakes from phloem elements of the host plants through a specific structure called haustorium. In France, its recent adaptation to winter oilseed crops (WOR, *Brassica napus* L.) results in an emergent agronomical problem causing severe yield losses. Our study aimed to give a better understanding of some functional traits of this new host-parasite interaction. Given that fertilization plays a major role in WOR productivity, our studies focused on nitrogen fluxes within host-parasite relationships. Comparative analyses were performed between two WOR accessions, ES Aliénor (Euralis Semences Company) and Shakira (Maïsadour Semences Company), which support rapid and slow emergence of the attached parasites in fields, respectively. Both unparasitized and parasitized WOR plants were grown during 6 months in controlled conditions. Once WOR plants were 6-week old, they were vernalized for 8-weeks. Vernalized plants were transferred back in greenhouse and supplied with ^{15}N-nitrate during a short 24 h-period. The different organs of unparasitized and parasitized WOR plants and attached broomrapes were harvested periodically during post-vernalization for N content and isotopic ratios (^{14}N/^{15}N) analyses by Isotopic Ratio Mass Spectrometry in addition to biomass determination. In parallel, kinetics of broomrape attachments was analyzed. Although unparasitized Shakira and ES Aliénor plants developed a similar total biomass, Shakira plants displayed a delay in pod setting. In addition, when challenged with *P. ramosa*, behavior of Shakira is characterized by a delay in broomrape attachment. Higher susceptibility to *P. ramosa* of pre-vernalized ES Aliénor plants favored early development of an important broomrape compartment of which biomass quickly equaled host biomass after vernalization. This was accompanied by differences in kinetics of ^{15}N allocation to broomrape attachments during the first week after vernalization within the two WOR genotypes. Stem development of both WOR accessions was significantly reduced under infestation. In addition, no tolerance was observed in ES Aliénor plants which did not set pods whereas Shakira plants displayed only a 50%-reduction in number of pods when infected. We hypothesize that delay in pod setting, in addition to reduced N flux to attached broomrapes of which development was ended, supported pod development in parasitized Shakira plants. Analysis of free ^{15}N-amino acid patterns from WOR leaf phloem exudates and broomrape organs is in progress in order to better characterize N fluxes within these interactions. This study gives information on tolerance mechanisms in WOR towards *P. ramosa*. It is financially supported by a scholarship from the "Ministère de l'Enseignement Supérieur et de la Recherche" (France) and funds from Maïsadour-Semences Company.

UNRAVELING THE MECHANISM INVOLVED IN *STRIGA* PARASITISM IN CEREALS UNDER NUTRIENT DEFICIENT CONDITIONS

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Striga hermonthica (*Striga*) is an obligate hemi-parasitic weed, causing severe yield losses in cereals throughout sub-Saharan Africa (SSA). Even though many possible explanations have been given for the *Striga* suppressing effect of highly fertile soils, the exact mechanism of this effect is unknown. We are interested to find out to what extent the strigolactones are involved in this phenomenon. The strigolactones are rhizosphere signalling molecules, biosynthesized in plants through the carotenoid pathway and secreted into the rhizosphere where they induce seed germination of parasitic plants. The amount of strigolactone production and *Striga* infection with various levels of N and P mineral nutrients, Urea, Triple Super Phosphate (TSP) and Diammonium Phosphate (DAP) fertilizers were studied in various cereal crops like rice, maize, sorghum and pearl millet. In addition to LC/MS analysis of strigolactones, *in-vitro* and *in-situ Striga* germination, attachment, and emergence rates were recorded. Results from the greenhouse and laboratory were further confirmed through field trials in Kenya and Mali. In all cereal crops, production of strigolactones was the highest under mineral deficient conditions, whereas increasing N and P dose reduced the amount of strigolactones in the exudates. Similarly, a steady reduction in *Striga* infection was observed with increasing levels of mineral nutrients. The present studies provide evidence that the extent of strigolactone production by the host plant decreased with increasing availability of nutrients in the soil concurring with decreased *Striga* incidence. The field results showed similar results although sometimes less convincing than the pot experiments. It is suggested that the effect of addition of nutrients to the soil on the reduction of *Striga* interacts with variety of the cereal, rainfall and background soil fertility levels. Our results show that the positive effect of N and P fertilizer application in *Striga* control is, at least partly, due to the suppression of strigolactone production and hence of *Striga* germination and subsequent attachment. Maintaining suitable N and P nutrient status of soil through wise use of fertilizers could be a promising strategy to reduce the negative effect of this noxious weed on cereal production in the African continent.

THE NATURAL GERMINATION STIMULANT OF *OROBANCHE CUMANA* IS NOT A STRIGOLACTONE

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The germination of obligatory root parasites of the Orobanchaceae depends on the perception of a chemical stimulus that is released from plant roots. This ensures that germination starts only when a root is available in the immediate vicinity of the seed, and increases the chance of the young seedling to connect and be nourished by a suitable host root. Several isoprenoid compounds, collectively termed strigolactones, stimulate the germination of various Orobanchaceae species but do not significantly elicit the germination of *O. cumana*, which is a specific root parasite of cultivated sunflower plants. Here we show that unlike the situation with other known *Orobanche* and *Phelipanche* species, the isoprenoid substance that is exuded from sunflower roots and exploited by *O. cumana* as its natural germination stimulant is not derived from the carotenoid biosynthetic pathway. Unlike the situation with strigolactone exuding plants, the stimulatory activity of sunflower root exudates markedly decreased under phosphate starvation. Mevastatin – an inhibitor of the cytosolic MVA pathway – blocked the release of the *O. cumana* germination stimulant in sunflower roots, while fluridone – an inhibitor of the carotenoid biosynthesis pathway – did not inhibit the release of the stimulant to the rhizosphere. These results indicate that the sunflower stimulant is not a strigolactone. The natural germination stimulant from sunflower root exudates was identified as dehydrocostus lactone (DCL), a guaianolide sesquiterpene lactone. Low concentrations of DCL from sunflower root exudates and from costus (*Saussurea lappa*) roots effectively stimulated the germination of *O. cumana* seeds but not of other weedy broomrapes like *O. cernua* and *P. aegyptiaca*. Differences were found in the germination response of various *O. cumana* populations to sunflower root exudates, which may indicate that similar to the variety of strigolactones that are found in root exudates of other plants, a variety of sesquiterpene lactones may be released by roots of various sunflower cultivars, and possibly also by roots of other plant species, in particular members of the Asteraceae where sesquiterpene lactones are common in plant tissues. Interestingly, no guaianolide sesquiterpene lactone was previously known to be exuded to the rhizosphere of any plant species. Unlike strigolactones, which are involved in the symbiotic recognition reaction of plant roots with soil microorganisms, no *raison d'être* is yet known for the excreted DCL that is exploited by *O. cumana* as a rhizospheric chemical signal for its germination. A defensive roles for this exudation may be a possibility, but mutual interaction with other organisms in the soil should also be considered for this and similar sesquiterpene lactones.

REPRESSING EFFECTS OF ARBUSCULAR MYCORRHIZAL FUNGI ON HAUSTORIAL INITIATION OF *PEDICULARIS TRICOLOR* (OROBANCHACEAE)

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Host mediated induction of haustorial initiation in root hemiparasitic plants have been widely and well studied. However, knowledge of other factors influencing haustorial development in parasitic plants has been very scarce. *Pedicularis tricolor* Hand.-Mazz. is a facultative root hemiparasitic species endemic to Southwestern China. Apart from its parasitic habit, *P. tricolor* has the potential to form mycorrhizal associations with arbuscular mycorrhizal fungi (AMF). In this study, we tested the effects of AMF on haustorial initiation of *P. tricolor* with a series of pot cultivation experiments, with or without host plants growing near the parasitic plants. Our results showed that AMF dramatically reduced haustorial numbers of *P. tricolor*, with or without the presence of host plants alike. While inoculation of AMF in the absence of *P. tricolor*'s host plants did not show significant effects on growth of the root parasite, severe growth-repressing effects were observed when *P. tricolor* was grown near a host plant otherwise proved to be favorable. The significant role of AMF in haustorial development of root parasitic plants was demonstrated for the first time. Also, the potential of AMF as biocontrol agents against root parasitic plants was discussed.

INDUCTION OF SEED GERMINATION IN *OROBANCHE* SPP. BY EXTRACTS OF TRADITIONAL CHINESE MEDICINAL HERBS

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Orobanche spp. is holoparasites of higher plants. Weeds of Orobanchaceae family are largely distributed in the Mediterranean region, western Asia and Eastern Europe, causing substantial losses to global crop production. They were considered the most important parasitic weed on a world wide scale. There are 11 species of the genus *Orobanche* in northern and southwestern China, especially in Xinjiang Uygur Autonomous Region, where it has caused yield losses of several economically important plants such as sunflower (*Helianthus annuus* L.), tobacco (*Nicotiana tabacum* L.), and tomato (*Lycopersicon esculentum* Mill). The co-evolution of *Orobanche* spp. and their hosts within the same environment has guaranteed a strong adaptation and effective parasitism. Scientists have focused on isolation and identification of germination stimulants from their hosts. Based on circumstantial evidences and experimentations, it was concluded that the germination stimulants from susceptible hosts and their synthetic analogues were likely to be unstable in the soil. Our objective was to explore non-host plants, namely, Chinese medicinal plants for potent, more stable stimulant production because of the medicinal plants could deal with human and animal diseases, in which they should containing active substances. Root extracts of 606 Chinese medicinal herb species were extracted with distilled water and methanol, and the extracts used for inducing germination of three *Orobanche* species, namely *O. minor*, *O. cumana* and *O. aegyptiaca*. *O. minor* exhibited a wide scope of germination across herbal extracts. *O. cumana* and *O. aegyptiaca* showed intermediate germination with the herbal extracts. *O. minor* with a broad host spectrum germinated better in different herbal extracts than *O. cumana* which have a narrow host spectrum and are more restricted to their host range. Methanol extracts of many Chinese herbal species were effective in stimulating seed germination among *Orobanche* spp. even though they are not as the typical hosts. This represents interesting examples of potential trap crops. In many countries of the world, people understand that herbs are beneficial to animals and humans. It is important for scientists in different countries of the world to screen their own herbaceous plants for possible induction of germination of *Orobanche* spp. seeds and use same as a trap crop, thereby diminishing the seed bank of *Orobanche*.

GERMINATION OF BROOMRAPE SEED ON DIFFERENT NUTRITIVE MEDIA

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In the agroecological conditions of Serbia broomrape (*Orobanche cumana*) as a parasite of sunflower has been appearing with varying intensity almost every year and can cause significant damage. Resistant or tolerant sunflower hybrids are the most efficient and the most economical measures in the suppression of this parasitic plant. In the tests of sunflower hybrids susceptibility to broomrape under artificial infestation, high germinativity of broomrape seeds used for infestation. The aim of this paper is to evaluate influence of different nutritive media on broomrape seed germination including water agar (WA), water agar+giberellic acid (GA conc. 25 ppm) and water agar+trifender (T conc.1%) in the presence or not of 7days old sunflower roots, hybrid (NS-H-111). Trifender is a biological pesticide from *Trichoderma asperellum* acting as plant growth promoter with beneficial side effect to control soil borne pathogens when incorporated in soil. Seeds of *Orobanche cumana* were collected in the sunflower fields in Vojvodina (Senta and Vrsac) during 2009. Seed samples were kept in the fridge on 4°C for 10 months for break dormancy. Surface sterilized seeds (25 seeds in 4 replicates for each treatment) were put in Petri dishes with nutritive media with or without roots of 7 day-old sunflower plants and incubated at 25°C in the dark. Germination rate was determined every 7 days under dissecting microscope. Data were analyzed by ANOVA and Duncan test. Broomrape seeds from Senta germinated on each nutritive media containing sunflower roots. After 7 days the highest germination rates were determined on GA (23%), while rates were much lower on WA (3%) and T (0%). After 39 days germination rates increased a little and reached 30% on GA, 6% on WA, and 5% on T. The slowest germination rates were determined on T medium where seeds started to germinate after only 12 days. Germination rates on the same media without sunflower roots were under 5%. Broomrape seeds from Vrsˇac shared poor germinativity on nutritive media and it was under 4%. Germination rates were the lowest on nutritive T media, therefore the effect of this bio-pesticide on broomrape seed germination should be further investigated.

ANATOMY OF THE HAUSTORIUM OF TWO SPECIES OF *STRUTHANTHUS* MART. (LORANTHACEAE)

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Haustorium is the way that the parasite connects physiologically to its host. It has evolved many times in evolution that is why it is not a homologous structure among the many angiosperm groups. It is a very diverse structure with many functional stages and forms that are indistinctly called by authors as haustorium, haustorial system, primary or secondary haustorium, endofitic system, holdfast, sinker and others. So, a precise anatomical and ontogenetical interpretation is needed in each case to a correct reference and mapping of their distribution in angiosperms. In the case of Loranthaceae, Calvin & Wilson (2006) recognize four basic haustorial types in aerial members. The genus *Struthanthus* has only the 'epicortical root' type. The goal of the present work is to study and compare in details the haustorium of *S. vulgaris* and *S. flexicaulis* that are very common parasitizing urban trees in São Paulo. *S. vulgaris* is a small plant (up to 1m long) and has a major haustorial region, around the place where seed germinated spreading from this point some epicortical roots to some meters of the host stem. On the other hand *S. flexicaulis* has a complex and widespread stem that can cover all tree canopies, with hundreds of attachment points, short and hooklike epicortical roots with many holdfasts secondary haustoria. So, the material was fixated, infiltrated in paraffin and sectioned following standard anatomical methods. Both species had similarities in their anatomic structure. In both cases it is possible to see at the beginning of the development a bulb with columnar cells in the region close to the host surface and also the formation of a cavity, filled of cell wall material and products of bulb cells lysis. In a more advanced stage, we see the formation of an intrusive organ and his penetration in host tissues only in *S. flexicaulis*. This organ is formed by parenchyma and tracheary cells in differentiation. In *S. vulgaris* the bulb almost touches the host secondary xylem, there is no intrusive organ and the sinkers develop directly from the bulb towards the host vessels. So it is clear that the structures between the two species are very similar but have specific characters that need a very careful observation and definition of each morphological structure. In secondary haustoria of the studied *Struthanthus* species it is possible to identify only one bulb per holdfast, with one intrusive organ and one sinker.

TRAFFICKING OF MACROMOLECULES IN THE PARASITISM ASSOCIATION BETWEEN EGYPTIAN BROOMRAPE (*PHELIPANCHE AEGYPTIACA*) AND TOMATO (*SOLANUM LYCOPERSICUM*)

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Egyptian broomrape (*Phelipanche aegyptiaca*) is considered a severe threat to the Mediterranean agriculture especially in arable and vegetables crops. This obligate root parasite depends entirely on its host for all nutritional and water requirements, resulting in massive yield losses. Once the parasite is attached to the host's root, it becomes a strong sink and draws assimilates from the host plant. At present, limited information is available regarding the processes involved in the traffic of assimilates and macromolecules in the host-parasite association. Therefore, the objective of the current research is to characterize the trafficking dynamics of a phloem mobile protein between the host and the parasite. Host-parasite system used for addressing this goal included tomato plants artificially infected with *P. aegyptiaca* grown in petri-dishes. This system allows an *in-situ* monitoring of the root zone. *P. aegyptiaca* seeds pretreated with GR24 were placed along the host root (less than 0.5 mm). A transgenic tomato line that expresses a green fluorescent protein (GFP) under the *AtSuc2* promoter was used. This GFP is produced in the companion cells and can move long-distance via the phloem in a similar manner as other solutes. We have found that the parasite has established a phloem connection with the host roots as early as five days after germination, when the tubercle diameter was 0.23 mm in average. Our results indicate that the fluorescence intensity kinetics in the growing attachment during early stages of parasitism can be described by an exponential relation indicating source-sink movement of the GFP. Although the GFP protein is of a medium-size, its transport most likely resembles the trafficking kinetics of plant endogenous compounds such as assimilates and amino acids. These compounds translocation will be characterize in future studies.

EFFECT OF SOME PLANT EXUDATES ON GERMINATION OF TWO *CUSCUTA* SPECIES

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Cuscuta campestris (L.) Yunck. and *Cuscuta approximata* Bab. are two important parasitic weed species in Turkey. Plant exudates can inhibit or stimulate germination of seeds. This information can be used to set proper integrated weed management system which is economically viable and environmentally sound. *C. campestris* seeds were collected from several hosts such as *Polygonum aviculare*, *Xanthium spinosum*, *Xanthium strumanium*, *Solanum nigrum*, *Convolvulas arvensis*, alfalfa, sugar beet, grape wine in Izmir, Canakkale, Denizli, Uak and Manisa Provinces, *C. approximata* seeds from alfalfa in Van Province in September and October 2009. Seedling of crops, tomato, pepper, bean, cowpea, and squash, which are common species in the region were uprooted and root exudates were obtained. Experiments set in petri dishes with 50 cuscuta seeds in 4 replications. Germinated seeds were removed every other day and experiment was terminated at 21st day after setting. Cowpea exudates did not affect germination of both dodder species. The germination of *C. approximata* was not affected by squash or pepper exudates while it was stimulated by tomato exudates and inhibited by bean exudates. Tomato, pepper and bean exudates inhibited *C. campestris* germination about 35 % while squash exudates increased germination by 50 %. These results suggest that there is a need further experimentation to understand reasons of differences.

Session 5

MEANS FOR PARASITIC WEED MANAGEMENT
(EWRS SESSION)

Chair

Diego RUBIALES and Grama Nanjappa DHANAPAL

CHEMICAL CONTROL OF *CUSCUTA SUAVEOLENS* ON SUGAR BEET AND ALFALFA

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Cuscuta suaveolens is a species of dodder causes serious damage to alfalfa and sugarbeet. The objective of this work was to test the effectiveness of various amounts of glyphosate at various rates for cuscuta control on sugar beet, and of paraquat followed by application of glyphosate at various rates before the flowering stage for cuscuta control in alfalfa. The treatments were applied when the parasite reached advanced stage (full flowering – fructification) in sugar beet and paraquat was applied just after cutting and glyphosate treatments before crop flowering in alfalfa. For sugar beet, the results showed low effectiveness not exceeding 20% due probably due to late application of glyphosate. Applied at flowering - fructification stage, the glyphosate would be absorbed in small quantity. Applied twice, treatments at Cuscuta early stage (beginning flowering) would be considered. Cuscuta can be controlled in with paraquat applied just after the crop cutting followed by 75 to 100 g a.i/ha of glyphosate. No toxicity symptoms were observed.

CHEMICAL CONTROL OF BROOMRAPE (*OROBANCHE CERNUA* LOEFL.) IN TOMATO (*LYCOPERSICON ESCULENTUM* MILL.) IN *ALFISOLS* OF SOUTHERN INDIA

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A field experiment was conducted to asses the efficacy of chemicals to control broomrape in tomato in farmer's field at Hosakote village in Karnataka State during *rabi*-summer season under irrigated condition. Hitherto, *Orobanche cernua* was severely affecting tobacco cultivation in India and hand weeding is the only current practice to control and now, it poses a serious threat to tomato cultivation. In Karnataka, tomato is being grown in an area of 4.6 million hectares with a production of 1.3 million metric tonnes and with a average productivity of 20.9 t/ha. The experimental site was located at 13^0 4′ N latitude and 77^0 7′ E longitude with an altitude of 870m amsl. The soil of the experimental site was red sandy loam in texture with neutral pH (6.7), electrical conductivity was 0.29 dS/m and medium in organic carbon (0.50%). Soil was low in available Nitrogen (210kg/ha), medium in available phosphorus (29.6kg/ha) and potassium (260kg/ha). The average normal rainfall at the site was 695mm.The experiment was laid out in Randomized Complete Block Design and replicated thrice. The treatments comprising four herbicides applied at two levels viz., sulfosulfuron (75% WG), glyphosate (41% SL), imazethapyr (10 WSC) were applied at 50 and 75g a.i./ha each of the treatment and metribuzin (70% WP) was applied at 150 and 250g a.i./ha, weed free check and weedy check. Herbicides were applied at 30 days after transplanting tomato. The broomrape spikes started emerging above-ground from 43-58 days after transplanting tomato and it took 37-50 days to complete its life cycle after emergence above-ground in tomato transplanted fields. Sulfosulfuron @ 75g a.i./ha recorded significantly lowest broomrape number, spike height and dry weight with higher broomrape control efficiency, which resulted in higher plant height, number of branches and leaf area per plant in tomato at harvest. Among all the chemical control treatments, sulfosulfuron @ 75g a.i./ha recorded higher fruit weight per plant and fruit yield of tomato. Tomato fruit yield had positive and significant correlation with number of fruits per plant (r=0.95), mean fruit weight (r=0.95) and the broomrape dry weight (r= - 0.80), broomrape spike number (r= - 0.83) and spike height (r= - 0.69) were negatively and significantly correlated with fruit yield at harvest. The benefit cost ratio was maximum in the weed free check (3.73) followed by sulfosulfuron @ 75g a.i./ha (3.67). In heavy infested broomrape fields, application of sulfosulfuron @ 75g a.i./ha is effective in preventing the development of broomrape and reduces the seed inoculum potential in the soil.

ARE WE MODELING THE MATH OR THE BIOLOGY OF PARASITISM DYNAMICS?

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During its initial stages of parasitism, the broomrapes (*Orobanche* and *Phelipanche* species) grow underground. Predicting their developmental stages at this phase is a necessity in order to properly apply control measures. This challenge can be met by using the modeling approach, as reported for *P. aegyptiaca*, *O. minor* and *O. cumana*, in tomato, red clover and sunflower fields, respectively. In those studies, the relations between parasitism dynamics and thermal time has been described by mathematical functions, e.g. sigmoid, logistic, Weibull, and polynomial functions. However, the mathematical functions should be carefully chosen, because in some cases the biological interpretation of the equations may be wrong even if great fit has been achieved, and then the goal of using curve fitting may be missed. Four years of field studies, conducted in various locations in Israel under different climatic conditions, resulted in a robust thermal time model that allows precisely predicting the parasitism dynamics. This was achieved by *in-situ* non-destructive monitoring and recording of the subsurface development of *O. cumana*, using the minirhyzotron. The data from field experiments, conducted in 2006, 2007, 2009 and 2010, were used for model calibration. For validation of the model, five field experiments were independently performed in 2009 and 2010. To estimate the number of attachments related to thermal time, the following equations were tested: Sigmoid, Gompertz (both three parameters) and Weibull (four parameters). These equations are characterized with the pattern lag, and with the log and maximal asymptote for the number of parasite tubercles as a function of thermal time. Fit of equations was evaluated by root mean-square error (RMSE), and by the corrected Akaike Information Criterion (AIC). In the calibration studies, the number of attachments was best fitted to thermal time using the Weibull equation, which resulted great fit in the validation studies (RMSE = 0.066; R^2 = 0.99; slope a ~ 1). In conclusion, the Weibull equation adds a biological dimension to the model, compared to the other equations, as the lag phase allows to estimate the precise timing of parasite attachment to host roots and of its flowering above soil surface. This information is crucial in any attempt to develop control strategies for these parasitic weeds.

INTEGRATED APPROACH FOR ALLEVIATING BROOMRAPE DAMAGE IN ISRAELI AGRICULTURE: A MULTI-DISCIPLINARY NATIONAL PROJECT

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Broomrapes (*Orobanche* and *Peliphanche* spp.) cause severe damage to vegetables and field crops worldwide. In Israel, during the last decade, the significance of infested fields has increased dramatically, causing heavy damage or even total yield losses. Egyptian broomrape (*P. aegyptiaca*) is the most abounded and devastating species throughout Israel, parasitizing wide host range. Hence, a combined efforts of the Israeli ministry of agriculture, the farmers' organizations and several research institutes for a three year-project (2010-2013) was established, aiming to manage broomrape in Israel agriculture. The project identified the broomrape as a national significance pest with the objective to provide the farmers with an integrated management of economical crop production in infested fields. The research and development project is composed of nine research teams that investigate topics related to both applied and basic aspects including phyto-sanitation and regulation. *P. aegyptiaca* in tomatoes and carrots serves as a host-parasite system. The specific topics include: a) developing a decision support system, for rational control with herbicides in carrots and tomatoes based on phenological model of *P. aegyptiaca*; b) detection and quantification of broomrape seed inoculum potential (seeds) in the soil; c) geographical information systems (GIS) mapping; d) soil disinfestations and adoption of effective measures; e) phyto-sanitation and seed disinfection of machinery and equipments, broomrape-free compost, and regulation; f) physiological aspects related to host-parasite relationship; g) optimization of herbicides action in plants and soil and h) remote sensing of broomrape. This interdisciplinary project may provide an integrated approach for managing broomrape on a wide scale of the intensive agriculture in Israel.

THE USE OF ARBUSCULAR MYCORRHIZAL (AM) FUNGI CONTROLLING *OROBANCHE MINOR* IN RED CLOVER (*TRIFOLIUM PRATENSE*)

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Orobanche minor is a native to the Middle East, but now found and classified as an invasive weed in the North-western region of the United States infesting mainly red clover as it is found outside its native range. It was thought to be brought into the States through seed contamination that was imported. A greenhouse experiment was conducted using arbuscular mycorrhizal (AM) fungi (*Glomus mosseae* – OR 218, *G. intraradice* – OR 216, *G. intraradice* (Commercial), *Acaulospora scrubiculata* – INVAM CL152, and *Scutellospora calospora* – OR 219 to evaluate their potential to control *O. minor* in red clover (*Trifolium pratense*). These treatments were compared to a control treatment where AM-fungi was not applied to clover infested with *O. minor.* The experiment was repeated twice in a randomized complete block design (RCBD) replicated six times. *Acaulospora scrobiculata* reduced emergence and total dry matter of *Orobanche*/clover plant. However, this treatment had the greatest number of *Orobanche* tubercles/clover root and produced the tallest clover plants and a greater total dry matter yield/clover plant than the control treatment. *Glomus mosseae* treatment prevented more *Orobanche* emergence with the least number of tubercles/clover plant, produced taller clover plants and a higher dry matter yield/clover plant than the control treatment. The per cent reduction of *Orobanche* emergence by *A. scrobiculata* and *G. mosseae* was 51.4% and 31.7%, respectively resulting in an increase of 40.5% and 17.5% in total clover dry matter yield/clover plant compared to the control treatment, respectively. Root colonization of clover by AM-fungi appears to correlate positively with the control of *Orobanche* as the higher the colonization of clover roots by the AM-fungi the better the control of *Orobanche* which resulted in better growth and greater red clover biomass. This study demonstrated the potential of AM-fungi to reduce/control or to compensate for *Orobanche* infestation and could be important for soil management, especially outside its native origin where the soil may be devoid of the right fungus to suppress its infestation.

STRIGA THE BEWITCHING WEED: INTERDISCIPLINARY CONTEXT OF CONTROL

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There are varied and numerous control methods for *Striga* both adopted, proposed and in development. No one of these is a panacea. Effective control is influenced by many factors and through understanding these and the ease of application in farmers own hands enables sustainable, integrated packages and recommendations to be developed and promoted. At the International Institute of Tropical Agriculture, based in Sub-Saharan Africa, we have a large group of interdisciplinary scientists and partners working together to achieve effective long term control solutions for farmers in the semi-arid areas where *Striga* is most prevalent. Our scientists work with international and national scientists, agribusinesses, farmers and extension agents to develop products and packages that address the most pertinent needs of the region. Working as a team we and we attempt to combine strategies to provide durable solutions e.g. *Striga* and drought tolerant cowpea varieties with appropriate agronomy options. This presentation details the approaches we take from SNP discover to agronomy and biocontrol to breeding to identify promising strategies and the adaptive research we undertake to enable farmer-friendly control package formulation.

INTEGRATED MANAGEMENT OF *STRIGA HERMONTHICA* IN MAIZE IN THE NIGERIAN SAVANNAS

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Striga hermonthica is a parasitic weed that attacks maize, sorghum and other staple cereal crops and has long been considered one of the greatest biotic constraints to cereal production in Africa. Use of resistant or tolerant maize varieties, maize-legume rotation using trap crop that stimulate germination of *striga* and the application of nitrogen fertilizer are all effective in reducing infestation and damage. This paper reports on the use of nitrogen fertilizers and resistant varieties to reduce *Striga* infestation and damage. Furthermore, the paper reports on the use of a participatory research and extension approach in assessing the performance and scaling-up of integrated *striga* control packages in three agro-ecological zones in Borno State, Nigeria.

There were significant reductions in the number of emerged *striga* at 120 kg N/ha for the early varieties and at 60 and 120 kg/ha for the late varieties. The number of emerged *striga* only significantly differs among the late varieties. The grain yield of the early varieties was 144 higher at 60 kg N/ha and 192 % higher at 120 kg N/ha than without added Nitrogen. For the late varieties, the grain yield was 85 % higher at 60 kg N ha and 144 % higher at 120 kg N/ha than without added nitrogen. Among the early varieties, TZE COMP4 C3 and TZB-SR had yielded significantly lower grain yields than other varieties. Our results showed that, the application of 60-120kg N/ha to *striga* resistant or tolerant varieties may reduce damage and increase grain yield. Higher economic returns was obtained at N rates of 60-120 kg N/ha than the other doses. The participatory process which encourages close intervention between research, extension and farmers, involved 30 local communities and 228 farmers representing 193 farmers group in identifying problems and seeking solution to them. Results showed not only effective *striga* control but productivity 200%.The involvement of local farmers' and groups in the evaluation process, firstly, helped to confirm that *striga* control can be best achieved using soybean followed by *striga*-resistant maize varieties together with productivity-increased management practices and, secondly promoted farmer-to-farmer extension activities. A participatory adoption assessment exercise indicated widespread of adoption of new varieties and management practices, despite the need for increased labour. Great potential exists to scale out the results to similar areas of Guinea and Sudan savannas in West Africa region.

EFFECT OF ORGANIC SOURCES AND UREA ON *STRIGA* INFESTATION AND MAIZE YIELD IN WESTERN KENYA

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Striga hermonthica (Delile) Benth. and declining soil fertility are serious threats to sustainable food production in western region of Kenya. To overcome this problem, promising integrated soil fertility management strategies were evaluated on their effectiveness on reduction of *Striga* and improvement of maize yields. The effect of integrated use of urea and *Calliandra* or maize stover on maize yield and *Striga* infestation was assessed in a field experiment carried out on a clay loam Ferralsol in western Kenya. Urea and *Calliandra* or maize stover were combined in a way to supply N at 75 kg ha^{-1} from both sources in 0:0, 100:0, 80:20, 60:40, 40:60, 20:80, 0:100 ratios arranged in randomized complete block design (RCBD) with 12 treatments replicated four times. Maize hybrid (cv WS 502) was used as a test crop. Data on *Striga* infestation maize grain yield and total biomass were recorded at harvest for five consecutive seasons. Maize plants under the control and high levels of stover were most severely stressed by *Striga*. Urea combined with *Calliandra* in the ratio of 40:60, 80:20 or urea combined with maize stover in the ratio of 40:60 and 60:40 consistently lowered *Striga* infestation than other treatments in all seasons. Combinations of organic and inorganic N sources that provided N in synchrony with crop demand at critical stages of maize development were able to withstand *Striga* stress Number of ears per plant was a major yield component and accounted for (R^2=0.74) of the variation in grain yield under *Striga*. Reduction in maize grain yield and its components were more severe under moisture stress that occurred in short rain seasons. The highest maize grain yield of 3.0 t ha^{-1} was obtained in treatments receiving N from maize stover and urea in the ratio of 40:60 followed by 2.7 t ha^{-1} obtained in treatments receiving N from Calliandra and urea in the ratio of 60:40. The control gave the lowest grain yield of 1.4 t ha^{-1}. Maize grain yields increased with increasing levels of urea under maize stover treatments. Harvest index ranged between 0.31 and 0.37. The negative effects of *Striga* on grain yields were dependent on *Striga* density and season. The use of both organic and inorganic fertilizers effectively reduced *Striga* infestation and improved crop yields. The study showed that integrated nutrient management is an effective means of maintaining arable lands to meet food production and reduce *Striga* infestation.

COULD THE *DESMODIUM* 'PUSH-PULL' SYSTEM FOR *STRIGA* CONTROL IN AFRICA WORK ON *PHELIPANCHE RAMOSA* AND *OROBANCHE CRENATA*?

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Approximately 1% of all flowering plants are parasitic, representing over 3000 species in 16 plant families. Among the greatest biological constraints to food production in sub-Saharan Africa are the parasitic weeds, *Striga hermonthica* and *S. asiatica*. In Mediterranean countries, the parasitic weeds, *Phelipanche* spp. and *Orobanche* spp. are important constraints. The aim of this ongoing project is to determine whether the 'push-pull' control system developed by ICIPE for *Striga* in Western Kenya using the forage legume, *Desmodium intortum* (green-leaf) and *D. uncinatum* (silver-leaf), could be adapted for control of *Phelipanche* and *Orobanche*. The roots of these *Desmodium* spp. exude two allelochemicals that influence the growth and development of *Striga*. One allelochemical stimulates seed germination. The key element is that a second allelochemical exuded from the *Desmodium* roots prevents attachment of the *Striga* haustorium to the roots of the maize host. Do root exudates from *Desmodium* spp. have analogous effects on *Orobanche* parasitism?

This question is being answered by a series of experiments. In a glasshouse experiment, water from pots containing *Desmodium* plants on an upper bench is being used to water pots containing host plants on a lower bench. This is achieved by watering the *Desmodium* to excess and piping the water which has passed through the *Desmodium* pots to the pots containing the host plants. Systems being tested include pea with *Orobanche crenata*, tomato with *Phelipanche ramosa* and, to test the system, millet with *S. hermonthica*. Both silver-leaf and green-leaf *Desmodium* species are being tested.

With appropriate dilution, exudates of both *Desmodium* species do indeed stimulate seed germination of *O. crenata* and *P. ramosa*. Results from the pot experiment suggest there may be some reduction in attachments of *P. ramosa* on tomato, but results obtained so far for *O. crenata* on pea are inconclusive. Additional experiments are underwayto determine any allelopathic effect of these allelochemicals or others exuded by the *Desmodium* on the crop plants. A major problem is of course that *Desmodium* spp. are not drought-tolerant and so would not be expected to survive in a Mediterranean climate. The possible application of root exudates via an irrigation system would therefore need to be considered.

MANAGEMENT OF BROOMRAPE AND DODDER USING NATURAL PLANT METABOLITES

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In the present study, the extracts of 10 Mediterranean plants were evaluated for herbicidal activity on early growth stages of field dodder (*Cuscuta campestris* Yuncker.) and two broomrape species (*Phelipanche ramosa* L. and *Orobanche crenata* Forsk.). The plant materials were collected from several sites in the western coastal part of Algiers, Algeria in March 2009. Aerial parts of each plant species were dried at shade at room temperature and reduced to powder using a blender. The powdered material was used for extraction along with methanol, ethyl acetate and dichloromethane (DM), and extracts were concentrated to dryness using a rotary evaporator at 40-48°C. The DM extracts were further analyzed by TLC. Extracts were chromatographed by TLC. Dried crude extracts, and chromatographic fractions were dissolved in methanol and used for bioassays on seed germination of the parasitic weed species. The results showed that the dichloromethane crude extracts were the most efficient. They either significantly reduced seed germination of *Cuscuta* (0 to 8% vs 37.5% for the control) or greater affected seedling length and emergence (95% and 53% reduction of seedling length and emergence, respectively compared to the control). When assayed on *Phelipanche* and *Orobanche*, about half of the extracts tested completely inhibited seed germination of *P. ramosa* and *O. crenata* and reduced by up to 94% the germ tube elongation of *O. crenata*. Overall, extracts from *Lavandula dentata*, *L. multifida*, *L. stoechas, Marrubium vulgare* and *Inula viscosa* were the most efficient and were still very active at 10-fold and 100-fold lower concentrations. Partial purification of the metabolites from effective DM extracts yielded 78 fractions, of which 36 exhibited strong antagonistic activity when assayed against *C. campestris*. Active compounds are being purified and characterized. Based on our findings, it can be concluded that metabolites from specific Mediterranean plant species have great potential as "natural herbicides" for parasitic weed management programs. Their merits as alternatives to synthetic chemicals may constitute a framework for future research axis.

Session 6

STRIGOLACTONES

Chair

Koichi YONEYAMA and Antonio EVIDENTE

STRIGOLACTONES AND BIOCONTROL AGENTS

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Several germination stimulants for *Orobanche* and *Striga* spp. have been identified in root exudates of their hosts and also in non-hosts. Most of them belong to the strigolactone family. Interestingly, the strigolactones act not just as germination stimulants for root parasitic plants, but some of them have recently been identified as the compound responsible for the induction of hyphal branching in arbuscular mycorrhizal fungi (AMF), which is a critical step in host recognition and for promoting their spore germination as well. Considering that strigolactones have been reported to be present in the root exudates of a wide range of plant species, it would not be surprising that these compounds act as signals not only for AMF but also for other organisms, both beneficial and damaging ones. To our knowledge only limited data have been available yet on the effect of strigolactones on other fungi apart from AMF, e.g. ectomycorrhizal fungi, beneficial fungi, soil-borne pathogens, as well as on other microorganisms living in the soil, e.g. rhizobacteria. Moreover, no information is available concerning the fate of the strigolactones released from the roots to the rhizosphere, e.g. how they are further metabolized by the soil microorganisms. These aspects could be highly intriguing from a practical point of view, offering novel tools or approaches for parasitic plant management, e.g.: plants could be protected from the attachment of parasitic weeds by using strigolactone-degrading microorganisms. Within a bilateral project between the Weed Science Center (Utsunomiya University, Japan) and the Institute of Sciences of Food Production (CNR, Italy) preliminary experiments have been carried out in order to evaluate the capability of microorganisms to metabolize the strigolactones, or conversely the influence of synthetic and natural strigolactones on the growth of microorganisms. For this purpose, two fungi having potential for biological control of *Orobanche (Phelipanche) ramosa* (one strain of *Fusarium oxysporum* and one of *Fusarium solani*) and one interesting biopesticide (a strain of *Trichoderma harzianum*) were used in the experiment. Fungi were grown in the presence of the strigolactones, and the reduction in strigolactone's content in the growth media during the incubation was determined by using high performance liquid chromatography-tandem mass spectrometry (LC-MS/MS). The fungi were also exposed to different concentrations of strigolactones in order to evaluate their possible influence on conidia germination and hyphal elongation. The very preliminary results will be presented at the Congress.

SYNTHESIS AND BIOLOGICAL ACTIVITY OF FLUORESCENT ANALOGUES OF STRIGOLACTONES

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Strigolactones are a group of terpenoids produced by the roots of plants, which induce germination of the parasitic weeds *Striga* (witchweed) and *Orobanche* (broomrape), as well as hyphal branching of arbuscular mycorrhizal (AM) fungi. Moreover, the recent discovery of an endogenous activity for strigolactones in the regulation of shoot branching in plants shows that they act not only as signalling molecules for parasitic and symbiotic interactions, but also as hormones, thus widening the action spectrum of strigolactones in plants. Recently, in our laboratory, a new class of conjugated strigolactone analogues, endowed with fluorescent properties, has been synthesized. The fluorescent behaviour of these new molecules could allow their application as intramolecular probes for bioimaging, with the final aim of identifying the structure and the location of the SL's receptor. Detailed knowledge of the receptor protein would provide insight into the mechanisms that are responsible for the biological effects, moreover it would enable the design of perfectly fitting non-natural stimulants or inhibitors that may be used to control parasitic weeds. The new strigolactone analogues were synthesized according to a simple and easily feasible procedure as a diastereomeric mixture or a racemic one, and the various isomers were separated, in order to elucidate the configuration-activity relationships. Bioassays using *Orobanche* seeds revealed that all these new SLs analogues stimulate germination, some of them at lower concentrations were more active than the standard stimulant GR24. In this contribution we will report the fluorescence spectra and the biological activity of these new molecules.

HOW DOES *STRIGA HERMONTHICA* ALTER THE GROWTH AND MORPHOLOGY OF RICE PLANTS; ARE STRIGOLACTONES INVOLVED?

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Striga hermonthica is an obligate, hemi-biotrophic root parasite that alters the growth, morphology and yield of the staple cereal crops (rice, maize, sorghum and millet) of sub Saharan Africa. Following germination of *Striga* seeds, in response to strigolactones present in host root exudates, the parasite radicle differentiates to form the haustorium which attaches to the host root. A wedge shaped file of cells then grows through the host root cortex and endodermis and the parasite establishes direct parasite-host xylem connections allowing withdrawal of water, organic and inorganic solutes. Within a few days of parasite attachment the host becomes visibly stunted and the stem appears thin in comparison to the uninfected plant. In cereals such as rice tiller emergence is delayed and the total number of tillers is substantially lower on infected compared to uninfected plants. As these changes occur so soon after attachment, when parasite biomass is very small, they are unlikely to be due to competition by the parasite for host resources. Recently it has been shown that strigolactones are involved in the control of shoot branching and in the regulation of tiller bud emergence in rice. In this study we investigate the hypothesis that infection of rice plants by *S. hermonthica* alters strigolactone metabolism leading to suppression of tiller bud emergence. We have (1) carried out a detailed analysis of parasite-induced changes in the morphology of rice plants, (2) determined how the expression of key genes involved in strigolactone metabolism alter in different host tissues following infection and (3) investigated the potential role of these hormones on the susceptibility of rice plants to the parasite. In order to determine when and how strigolactones are altered in different regions of the infected plant we infected a transgenic rice line containing the strigolactone biosynthetic gene CCD8 (carotenoid cleavage dioxygenase 8) fused to the GUS reporter gene and profiled the expression of key genes involved in strigolactone biosynthesis, degradation and signalling by qRT-PCR. Finally we examined the effect of increasing or decreasing the concentration of strigolactones (by the application of the carotenoid biosynthetic inhibitor fluridone or by adding the synthetic strigolactone GR24, respectively) on tillering in infected and uninfected plants and on susceptibility to the parasite. We have shown that the genes encoding strigolactone biosynthesis are upregulated and/or down regulated both temporally and spatially in discrete regions of the plant at key stages of the parasite life cycle. For example, upregulation of CCD8 was observed in cells surrounding tiller buds in *Striga*-infected plants when compared to uninfected controls consistent with a role in the suppression of the emergence of tillers in the infected plant. Interestingly, strigolactone biosynthesis was also upregulated and then down regulated in a very precise spatial and temporal manner in host roots during the early stages of infection. These findings will be discussed in the context of the potential roles of strigolactones post attachment in this complex host-parasite interaction.

TARGET SELECTIVE STRIGORACTONE ANALOGUES

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Recent studies of highly branched mutants figured out that strigolactones (SLs) work as a phytohormone inhibiting shoot branching. When SLs are exogenously applied to SL biosynthesis mutants such as rice *d10-1*, they inhibit the shoot branching and restore its highly branching phenotype to that of wild type. Nowadays application of SLs to agriculture is highly expected to protect crops against damages by parasitic plants. Until now, several SL analogues targeting parasitic plants have been synthesized, but they have defects in their application to agriculture: low stability in circumstances due to the existence of labile enolether moiety and high cost for their chemical synthesis. To solve these problems we have been attempting to prepare highly active SL-mimics in short synthetic pathway. Among synthesized 5-functionalized 3-methylfuran-2(5H)-one derivatives possessing a new chemical skeleton, 5-(4-chlorophenoxy)-3-methylfuran-2(5H)-one, synthesized in one-pot reaction with phenol and bromofuranone, was found as a good lead compound for mimicking SL action. So, we prepared several derivatives based on this compounds to examine their activity for the inhibition of shoot branching of rice *d10-1*. Because SLs also show the activity for inducing seed germination of parasitic plants, the induction activity of these chemicals was also evaluated. Here we report the structure-activity relationships of these compounds and their target selectivity.

TRANSCRIPTOME ANALYSIS OF THE PARASITIC PLANT *PHTHEIROSPERMUM JAPONICUM*

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Parasitic plants attack economically important crops. They invade host roots to take water and nutrients through specialized structure, the haustorium. In contrast to their economical impact, it is widely unknown how parasitic plants invade the host roots. The facultative parasite *Phtheirospermum japonicum* has been applied as a model for parasitism studies because it can be genetically manipulated and due to its kinship with noxious parasitic plants. To find the genes essential for parasitism the transcriptome of *P. japonicum* on parasitic or non-parasitic stages were sequenced. To maximize the gene discovery and facilitate the *de novo* transcriptome assembly the sequencing analysis was carried out using two platforms, Roche 454 and Illumina. The hybrid assembly resulted in 58137 contigs with minimum size of 300 bp. Out of them, 4746 sequences were exclusively found in the parasitic stage and 1445 showed 10 times higher expression in the parasitic tissues compare with the control. The gene ontology analysis revealed that genes in the category of "structural molecules" or "hydrolase activity" are enriched in the transcripts from parasitic stage. The sequences classified as "structural molecules" include genes involved in protein synthesis, while many of those in "hydrolase activity" category are proteases. These data suggest that high protein synthesis and protease activity are recruited during the infection processes of *P. japonicum*. To further analyze the expression pattern during the infection process, the *P. japonicum* microarray (Agilent) was designed and hybridized with labeled cRNA extracted in different time points throughout the infectious process upon the host contact or upon the treatment with DMBQ (2,6-dimethoxy-*p*-benzoquinone), a chemical able to induce the haustorium *in vitro*. These results will contribute to understand which genes are essential to establish a parasitic interaction with the host.

GIBBERELLIN REGULATES STRIGOLACTONE BIOSYNTHESIS

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Root parasitic weeds are responsible for large-scale crop devastation all over the world. Seeds of root parasitic weeds initiate germination by recognizing secondary metabolites, strigolactones, which are derived from carotenoid and secreted from the roots of the host crops. Strigolactones are also rhizosphere signaling chemicals for symbiotic arbuscular mycorrhizal fungi, and it has been reported that strigolactones or their metabolites act as a novel plant hormone in shoot branching.

For the control of root parasitic weeds, use of strigolactone biosynthesis inhibitors is one of promising ways. To screen for the chemicals that inhibit strigolactone biosynthesis, we used chemical library constructed in our laboratory. Analysis by LC/MSMS revealed that gibberellin (GA) reduced the level of a strigolactone in roots of both rice and *Lotus japonicus*. In addition, GA treatment in rice also inhibited the *Striga* germination and infection. These results suggest that GA should be a new tool for controlling the parasitic weeds.

EFFECT OF LYOPHILIZATION ON EXTRACTION OF STRIGOLACTONES

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Strigolactones (SLs) are allelochemicals that act as germination stimulants for root parasitic plants and host recognition signals for arbuscular mycorrhizal fungi in the rhizosphere. In addition to these functions, SLs are now recognized as a novel class of plant hormones inhibiting shoot outgrowth. Although extensive studies on the isolation and structural elucidation of SLs have been conducted, SLs often decompose during purifications due to their chemical instabilities. We noticed significant reduction of SLs when root exudates or plant tissues were kept in a freezer. In this study, quantitative differences of SLs between fresh and freeze-dried samples were examined.

Houttuynia cordata Thumb., a Chinese medicinal plant, which had been reported to stimulate seed germination of *Striga hermonthica*, was collected on the campus of Utsunomiya university in Japan. Fresh and freeze-dried roots were extracted with ethyl acetate (EtOAc). After filtration, EtOAc solutions were washed with 0.2 M K_2HPO_4 to yield neutral EtOAc soluble (NE) fractions. NE fractions were dried over anhydrous $MgSO_4$ and evaporated *in vacuo* to afford crude samples. The crude samples were subjected to LC-MS/MS analysis and germination test using seeds of clover broomrape (*Orobanche minor*).

H. cordata was found to produce strigol, sorgomol, and 5 -deoxystrigol by LC-MS/MS analysis. The levels of SLs in the NE fractions from the freeze-dried sample were only 1/10 that of fresh sample, indicating that SLs were decomposed during the lyophilization. However, crude extracts of both fresh and freeze-dried samples showed a similar level of germination stimulating activity toward *O. minor*. These results indicate that SLs may not only be decomposed but also be converted to active compounds.

GERMINATION STIMULANTS FOR *STRIGA GESNERIOIDES* FROM COWPEA (*VIGNA UNGUICULATA*)

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Alectrol, a *Striga gesnerioides* germination stimulant, was reported from cowpea (*Vigna unguiculata*) root exudates. Alectrol, identified most probably as (+)-orobanchyl acetate, was also detected in root exudates of red clover. Germination of *S. gesnerioides* has strict stereochemistry requirements. The synthetic 4-hydroxy-GR24 (HO-GR24) and its acetate whose configuration is the same as that of strigol, induced negligible germination. On the other hand, HO-GR24 and its acetate, whose configuration is the same as that of fabacyl acetate, induced considerable germination. In the present study the germination stimulants for seeds of *S. gesnerioides* produced by cowpea were reinvestigated. Cowpea was grown hydroponically, under a phosphate-deficient condition, and root exudates were collected by absorption onto activated charcoal. The collected root exudates were subjected to solvent partitioning to give a neutral EtOAc fraction, which was fractionated by column chromatography on silica gel with a mixed solvent of hexane and EtOAc. Two germination stimulants, **1** and **2**, eluted with 50% and 70% EtOAc, respectively, were detected. The ESI-MS analysis of the less polar stimulant **1** afforded a proton adduct ion at *m/z* 389 along with a sodium adduct ion at *m/z* 411. The MS/MS analysis of the ion at *m/z* 389 gave fragment ions at *m/z* 347, 329, 233, 205 and 97, suggesting that the compound was orobanchyl acetate. However, LC analysis showed a retention time consistent with that of authentic 2'-*epi*-orobanchyl acetate, but inconsistent with those of authentic orobanchyl acetate, 4-*epi*-orobanchyl acetate, or 4,2'-*bisepi*-orobanchyl acetate. Similarly, the more polar stimulant **2** was identified as 2'-*epi*-orobanchol. The stimulants had a negative Cotton effect above 250 nm, thus suggesting an *R*-configuration in the D ring. Moreover, the CD curves of the stimulants are opposite to those of the corresponding (2'*S*)-isomers. Therefore, it was concluded that *S. gesnerioides* germination stimulants produced by cowpea are (2'*R*)-2'-*epi*-orobanchol and its acetate.

PROMOTIVE AND INHIBITORY STEREOISOMERS OF STRIGOLACTONES TO SEED GERMINATION OF *STRIGA GESNERIOIDES*

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Striga gesnerioides, a root parasitic weed of economic significance on cowpea, is widely distributed in Africa. A germination stimulant, designated alectrol, was isolated from cowpea root exudates. However, its true structure remains controversial. Recent work indicated that alectrol is most probably orobanchyl acetate. Based on insensitivity of *S. gesnerioides* seeds to the synthetic germination stimulant, GR24, the importance of an oxidized functional group in the B-ring of strigolactones, for induction of *S. gesnerioides* germination, was suspected. GR24, 4-hydroxy-GR24 (HO-GR24) and 4-acetoxy-GR24 (AcO-GR24) were synthesized and their potential as *S. gesnerioides* germination stimulants was evaluated. However, they proved to be inactive. The respective stereoisomers were separated and evaluated for germination-inducing activities. The stereoisomers of HO-GR24 and AcO-GR24 having the configuration 3a*R*,8b*R*,2'*R* were active, while GR24 stereoisomer, having the same configuration, was inactive. (3a*S*,8b*S*,2'*R*)-HO-GR24 and its acetate, whose configuration is the same as (+)-strigol, induced negligible germination. Furthermore, these compounds inhibited cowpea root exudates-induced germination of *S. gesnerioides*. Chromatographic separation of root exudates of hydroponically grown cowpea resulted in isolation of two germination stimulants for *S. gesnerioides* seeds. Mass spectral analyses suggested that the compounds were orobanchol and orobanchyl acetate. However, LC analysis showed retention times consistent with those of authentic 2'-*epi*-orobanchol and its acetate derivative. Moreover, the CD curves were symmetrical to those of authentic samples of the corresponding (2'*S*)-isomers. Therefore, it could be concluded that germination of *S. gesnerioides* has strict stereochemistry requirements and that the germination stimulants in cowpea root exudates are (2'*R*)-2'-*epi*-orobanchol and its acetate.

STRUCTURAL REQUIREMENTS OF STRIGOLACTONES FOR INDUCTION OF GERMINATION IN ROOT PARASITIC PLANTS

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In nature the seeds of the root parasitic weeds of the genera *Orobanche* and *Striga* only germinate when exposed to stimulants released from roots of host and some non-host plants. The stimulants are collectively referred to as strigolactones. A synthetic analog of strigolactones, GR24, induces germination of seeds of several root parasitic plants including *O. minor* and *S. hermonthica*, but not *S. gesnerioides*. Germination of the latter is only elicited by cowpea root exudates or alectrol, a strigolactone, isolated from its root exudates. The recent proposal that the true structure of alectrol is the same as that of orobanchyl acetate, suggests that a modification in the B-ring of the strigolactones is important for germination induction of *S. gesnerioides* seeds. In the present study, GR24, 4-hydroxy-GR24 (HO-GR24) and 4-acetoxy-GR24 (AcO-GR24), which have an oxidized functional group in the B-ring, were prepared and evaluated for ability to induce germination of *S. hermonthica*, *O. minor* and *S. gesnerioides* seeds. GR24 was more active on *S. hermonthica* than HO-GR24 and AcO-GR24. However, on *O. minor* seeds, HO-GR24 was the most active. In contrast, *S. gesnerioides* seeds did not respond to any of the three compounds, but were highly responsive to cowpea root exudates. Strigolactones of plant origin are, exclusively, optically active with chiral centers in the tricyclic system and the D ring, while the synthetic strigolactones used in this study are diastereomeric and racemic mixtures. The respective stereoisomers were chromatographically separated. The stereoisomers of HO-GR24 and AcO-GR24, having the same configuration as fabacyl acetate, were effective germination stimulants for *S. gesnerioides*. On the other hand, HO-GR24 and its acetate derivative, having the same configuration as strigol, induced negligible germination. HO-GR24 and its acetate derivative having the same configuration as strigol and four GR24 stereoisomers were inhibitory to cowpea root exudates-induced germination of *S. gesnerioides* seeds. The results suggested that both an oxidized substituent at C-4 and the configuration of the tricyclic lactone and the D ring are essential structural requirements for induction of germination in *S. gesnerioides* seeds.

GERMINATION STIMULANTS FOR ROOT PARASITIC PLANTS PRODUCED BY *LINUM USITATISSIMUM* L.

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Broomrapes (*Orobanche* and *Phelipanche* spp.) and witchweeds (*Striga* spp.) are obligate destructive parasitic plants infecting roots of many economically important crops. The lifecycle of these parasites is closely associated with their hosts, and secondary metabolites produced by host roots play an important role in the host-parasite interaction. Seeds of these parasites germinate only when they are exposed to strigolactone (SL) germination stimulants produced by and released from roots of plants. So far, 16 SLs have been isolated as germination stimulants for the root parasites. In this study, characterization of SLs produced by flax (*Linum usitatissimum* L.) which had been reported to produce large amounts of stimulants (Brown et al., Biochem. J. 48: 559, 1951) was conducted.

Approximately 5,000 flax seedlings were grown hydroponically and root exudates collected. The root exudates were subjected to solvent partitioning to give a neutral ethyl acetate (EtOAc) soluble fraction. This was purified by a silica gel column chromatography eluted with *n*-hexane–EtOAc and a series of reversed phase HPLCs for the germination stimulant activity on *O. minor* seeds. In addition, these extracts were directly analyzed by using HPLC linked with tandem MS (LC-MS/MS) to detect known SLs and their isomers.

The root exudates of flax were found to contain at least 11 different SLs including orobanchol, orobanchyl acetate and 5-deoxystrigol by LC/MS/MS and GC/MS analyses. Six novel SLs were purified and determined as 7-oxoorobanchol, 7α-hydroxyorobanchol, 7ß-hydroxyorobanchol, and their acetates by spectroscopic analyses.

CHARACTERIZATIONS OF STRIGOLACTONES IN PLANT CELL CULTURES

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Root parasitic plants and arbuscular mycorrhizal fungi receive strigolactones (SLs) as host recognition signals in the rhizosphere. In host plants, SLs play a key role in shoot branching. Several lines of genetic evidence have illustrated that SLs are derived from the carotenoid pathway in plants. However, the biosynthesis pathway of SLs has not yet been fully elucidated. Establishment of their biosynthesis pathway and subsequent studies on regulation of their biosynthesis will provide important clues to control their physiological functions. To better understand the biosynthesis of SLs in plants, we investigated the occurrence of SLs and regulation of their biosynthesis in plant cultured cells.

Suspension cell cultures of Arabidopsis and rice were provided from RIKEN BioResource Center of Japan. The suspension cell cultures were separated into cells and culture media before their stationary phase. The neutral ethyl acetate-soluble fractions were examined by a bioassay using seeds of root parasitic plant *Orobanche minor*. Remarkable activities on the germination stimulation were found in both the cells and culture media of Arabidopsis and rice.

In order to know whether the germination stimulants found in the cell cultures are SLs, the extracts from cells and culture media of Arabidopsis and rice were analyzed by LC-MS/MS. It was shown that both the cells produced orobanchol, orobanchyl acetate, 7-hydroxyorobanchyl acetate and 7-oxoorobanchyl acetate and released these SLs to the culture media. SLs, however, were produced transiently in the cultured cells, suggesting that the production of SLs may be regulated by a feedback from own level or growth conditions including cell density. The efflux as well as production of SLs by the cultured cells was altered by phosphate starvation. When the cultured cells were grown in a phosphate-deficient medium before or after the increase in SL yield of the cultured cells, the production or efflux of SLs was promoted, respectively. This finding indicates that plant cell cultures would be a good system for understanding the exudation mechanism of SL.

Furthermore, an experimental proof for an SL conversion was first obtained using the plant cell cultures. Deuterium-labeled 5-deoxystrigol was converted into the labeled orobanchol and several unknown oxidized metabolites in the cultured cells. Feeding experiments using plant cell cultures would shed light on unknown pathways in the SL biosynthesis.

NEW STRIGOLACTONE ANALOGUES, DESIGN, SYNTHESIS AND BIOACTIVITY

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Over the years our research program focused on the chemistry and structure-activity relationship of germination stimulants for parasitic weeds. The most common stimulants are the so-called strigolactones (SLs). Earlier we synthesized the naturally occurring SLs, sorgolactone, strigol and orobanchol, and the SL analogues GR 24, GR 7 and Nijmegen-1. In all cases we prepared all possible stereoisomers and assayed their activity towards seeds of *Striga* and *Orobanche*. In this communication we report on the synthesis of aromatic orobanchol, which is the natural stimulant wherein the A-ring is replaced by an aromatic ring. This synthesis could be accomplished by a selective oxidation of the B-ring in the ABC moiety of GR 24. After coupling with the D-ring requiring an unexpected protection of the hydroxyl group, both epimeric B-ring hydroxy analogues were obtained. All racemic stereoisomers were bioassayed, as well as their acetates. The biodata reveal that the isomer with the natural configuration at all three stereogenic centers is most active and that hydrogen bonding of the B-ring OH does not play an important role in binding of the SL in the receptor.

New SL analogues were designed using a general working model for germination stimulants as a blueprint. This model was derived by combining the structural features of the bioactiphore which resides in the CD part of the SLs and the molecular mechanism of action of SLs. A series of new SL analogues was thus prepared in a rather simple two step synthetic operation using readily available ketones, such as 1-tetralone, 1-indanone, various substituted cyclohexanones and naturally occurring carvone and pulegone, as the starting material. The biodata of these new SL analogues reveal that some of them are as active as GR 24.

A second series of new SL analogues was obtained in a single step synthesis starting from readily available keto enols, such as 4-hydroxycoumarin and dimedone. Most of these newly prepared SL analogues show a high germination activity towards *Striga* and *Orobanche* seeds, some of them even have an activity comparable to GR 24. An important conclusion is that the working model is applicable for the design of new SLs. The best performing new SL analogues are candidates for the use in the suicidal germination approach in combating parasitic weeds. Special attention will be given to stereochemical aspects of the bioactivity of SLs.

Session 7

CROP RESISTANCE TO PARASITIC WEEDS AND CROP BREEDING

Chair

Julie SCHOLES and Alejandro PÉREZ DE LUQUE

BREEDING *STRIGA* RESISTANT EARLY AND EXTRA-EARLY MAIZE HYBRIDS FOR WEST AFRICAN FARMERS

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Provision of food for the fast growing population of sub-Saharan Africa is a major challenge. Maize (*Zea mays* L.) has good prospects to rectify the food deficit in West Africa (WA) and the savannas have been identified as the 'Maize Belt' of this sub-region. However, infestation by *Striga hermonthica* (Del.) Benth. severely constrains maize production in this agro-ecosystem. This parasitic weed causes yield losses ranging from 20 to 80%; complete yield loss also occurs and many resource-poor farmers have been compelled to abandon their fields. Host plant resistance or tolerance is considered the most affordable and environmentally friendly for resource-poor farmers. Recognizing the significant negative impact of *S. hermonthica* infestation on maize adoption in the savannas of WA, the International Institute of Tropical Agriculture (IITA) has during the last three decades emphasized the development of source populations, open-pollinated varieties, inbred lines and hybrids that combine earliness or extra-earliness with tolerance or resistance to *Striga*, along with drought and low-N stresses. Backcrossing, inbreeding, hybridization, and the S1 recurrent selection method, along with screening under artificial infestation were used to develop the products. Three studies are reported here to demonstrate progress made so far. In the first study, diallel crosses of nine early maturing white maize inbreds were evaluated under drought, well-watered, *Striga*-infested, and *Striga*-free conditions at five locations in Nigeria in 2007-2009 to examine the combining ability, inbred per se performance and identify the heterotic groups. Additive gene action was more important in the inheritance of drought tolerance and *Striga* resistance. GGE biplot analysis revealed that TZEI 22, TZEI 4 and TZEI 5 were the most stable inbreds across test environments. Entry TZEI 7 had the highest GCA effects while TZEI 3, TZEI 5, and TZEI 19 had the highest SCA effects. Two heterotic groups were identified; TZEI 7, TZEI 19, TZEI 2, and TZEI 4, in the first group; TZEI 5 and TZEI 3 constituted the second. Inbred TZEI 3 was identified as the ideal tester across test environments. In the second study, 21 early maturing hybrids plus three checks were evaluated under *Striga*-infested and *Striga*-free conditions at Mokwa and Abuja, Nigeria in 2009. The top ranking hybrid, TZEI 2 x TZEI 87 out yielded the susceptible check TZE Comp 3 DT C1F2 by 68% under *Striga* infestation and 17% under *Striga*-free conditions. The hybrids TZEI 24 x TZEI 17, TZEI 8 x TZEI 17 and TZE-Y Pop DT STR x TZEI 17 were stable and high yielding while TZEI 24 x TZEI 17 and TZEI 2 x TZEI 87 were the closest to the ideal genotype under *Striga* infestation. In the third study, 136 extra-early hybrids plus 4 checks were evaluated under *Striga*-infested environments at Mokwa and Abuja in 2010 to identify the best performing and stable hybrids based on multiple trait selection. Under *Striga* infestation, TZEEI 4 x TZEEI 14 out yielded the early susceptible check, TZE Comp 3 C3 by 55%. In the three studies, several other stable, high-yielding *Striga*-resistant hybrids were identified. *Striga* resistant elite varieties have been released and widely adopted in Nigeria, Benin, Ghana and Mali. Parental lines of *Striga* resistant hybrids have been made available to seed companies for commercial seed production.

IDENTIFICATION OF NOVEL SOURCES OF RESISTANCE TO *STRIGA* IN THE AFRICA RICE SPECIES *ORYZA GLABERRIMA*

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Sorghum, maize and upland rice are staple foods for millions of people in sub Saharan Africa yet a major biotic constraint to crop production and yield improvement is the parasitic weed *Striga*. *Striga* attaches to the roots of the host plant causing severe stunting and yield losses of 40-100%. The use of *Striga*-resistant cultivars is recognized as a sustainable and cost effective control strategy. However, their use is limited by a lack of resistant germplasm and an understanding of the molecular genetic nature of host resistance to this parasitic weed. Our work is focussed on the discovery of novel sources of resistance in rice to *Striga* and the identification of resistance mechanisms at a molecular genetic level. The cultivated African rice species *O. glaberrima* and wild relatives of rice represent valuable sources of genetic variation that that could be exploited for the improvement of elite cultivars. As part of a Generation Challenge Programme Mathias Lorieux, IRD/CIAT and colleagues have introgressed segments of the genomes of the African and wild rice species into highly productive *O. sativa* cultivars to produce Chromosome Segment Substitution Line (CSSL) populations for genetic analysis of complex traits. The objectives of this study were (1) to determine whether cultivars of *O. sativa*, *O. glaberrima*, *O. barthii*, *O. meridionalis* and *O. rufipogon* (parents of CSSL populations) were susceptible or resistance to *S. hermonthica* and (2) to identify *Striga* resistance QTL using an *O. sativa* (Caiapo) *X O. glaberrima* (MG12) CSSL population. Rice plants were grown in rhizotrons and inoculated with pre-germinated *Striga* seeds. The African rice cultivar (MG12) and all the wild relative cultivars exhibited strong post attachment resistance to *S. hermonthica* whereas the *O. sativa* cultivars (Caiapo and Curinga) were very susceptible, an ideal situation for the identification of QTL and genes underlying resistance. To identify QTL for post attachment resistance we infected 64 CSSLs possessing contiguous chromosomal segments from *O.glaberrima* (cv. MG12) in the background of *O. sativa* (cv. Caiapo) with an ecotype of *S. hermonthica* collected from Kibos, Kenya. Most of the CSSLs were susceptible to *S. hermonthica* but two CSSLs showed the same resistance phenotype as MG12. QTL analysis revealed a highly significant QTL on chromosome 12. The data suggest that a substantial part of the resistance in MG12 to this ecotype of *S. hermonthica* is due to a small number of genes of major effect. We are currently carrying out further studies to fine map this region and identify the genes involved. To determine whether the resistance was broad spectrum or confined to one ecotype of *S. hermonthica*, Caiapo, MG12 and the two resistant CSSLs were evaluated for resistance to other *S. hermonthica* and *S. asiatica* ecotypes. The same pattern of resistance was observed for several ecotypes of both *Striga* species indicating that it is relatively broad spectrum in effect although the CSSLs were susceptible to two other ecotypes tested. However, in these cases the resistance of the *O. glaberrima* parent (MG12) was not compromised suggesting that other resistance genes exist at different chromosamal locations in the *O. glaberrima* genome. Our results indicate that the *O. glaberrima* and wild relative genomes are excellent sources of resistance to *Striga* and will allow the identification of resistance genes active against different ecotypes of the parasite.

POST-ATTACHMENT RESISTANCE OF INTERSPECIFIC NERICA RICE CULTIVARS TO THE PARASITIC WEEDS *STRIGA HERMONTHICA* AND *S. ASIATICA*

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New rice for Africa (NERICA) are a group of interspecific cultivars developed by Africa Rice Center and partners to combine the high weed competitiveness and good levels of resistance to abiotic and biotic stresses of the African rice species (*O. glaberrima*), with the high yield and grain quality of the Asian rice species (*O. sativa*). The NERICA cultivars are currently distributed and grown across sub-Saharan Africa (SSA). In many rain-fed upland locations *Striga* spp. are important production constraints in rice causing severe loss of yield. The use of *Striga*-resistant host cultivars is considered a key component of an integrated management strategy although little is known about the resistance of different NERICA cultivars to *Striga* species or ecotypes. The objectives of this study were to (1) determine which NERICA cultivars exhibit strong post-attachment resistance to *S. hermonthica* and *S. asiatica* (2) identify whether resistance was broad spectrum or specific to particular ecotypes of the parasites and (3) characterize the phenotype of the resistance at a histological level. To achieve our objectives, all 18 upland NERICA cultivars and their parents were evaluated for post-attachment resistance to different ecotypes of *S. hermonthica* and *S. asiatica* in rhizotrons at the University of Sheffield. NERICA 1 to 5 and NERICA 10 exhibited a resistance response that was superior to their resistant *O. glaberrima* parent CG14, while NERICA 6, 7, 8, 9, 11, 14, 15, 16 and 18 were more susceptible than their *O. sativa* parents (WAB 56-104, WAB 56-50 and WAB181-18). NERICA 12, 13 and 17 showed intermediate levels of resistance. The resistance phenotype was characterised by necrosis at the host-parasite interface and, in many cases, an inability of the parasite to penetrate the endodermis. The resistance ranking of the cultivars was similar for several ecotypes of *S. hermonthica* and also for the ecotype of S. asiatica from the USA. However, the pattern of resistance and susceptibility was not always the same; when NERICA 1 and 10, the most resistant cultivars, were infected with an ecotype of *S. hermonthica* collected from a farmer's field in Kouto, Côte d'Ivoire where NERICA 1 had been grown for several years, resistance broke down suggesting that virulence had evolved in this population. In addition, when some of the more susceptible cultivars were infected with an ecotype of *S. asiatica* collected from Kyela, Tanzania, where NERICA cultivars had not been grown before, good levels of resistance were observed. Our data suggest that some of the NERICA cultivars possess good resistance to a range of *Striga* ecotypes and could be recommended to farmers for use in *Striga*-infested fields. However, our results also indicate that greater knowledge of the molecular basis of both host resistance and host virulence is critical for the breeding of durable resistance (and pyramiding of appropriate resistance genes) in host cultivars adapted to different rice agro-ecosystems in sub-Saharan Africa.

CHARACTERISATION OF A NOVEL TOMATO MUTANT HRT-1 RESISTANT TO ACETOLACTATE SYNTHASE INHIBITING HERBICIDES FOR BROOMRAPE MANAGEMENT

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Orobanche and *Phelipanche*, commonly known as broomrape, are dicotyledonous holoparasitic flowering plants that cause heavy economic losses in a wide variety of plant species. In Israel *P. aegyptiaca* is the most destructive pest on tomato. Acetolactate synthase (ALS) inhibiting herbicides and glyphosate are the only herbicides known to control broomrapes. ALS inhibiting herbicides inhibit the enzyme acetolectate synthase, a key enzyme in the branched chain amino acid biosynthesis pathway leading to the formation of leucin, valin and isoleucin. Pulsar and Pursuit, imidazolinone herbicides, are in use for broomrape management in clover and pea, respectively. Tomato plants are very sensitive to these herbicides, while herbicides from the sulfonilurea group may be used for broomrape control in tomato. But their application is based on a complicated protocol that includes 5 herbicide applications in a precise timing based on growing degree days model. Each application should be followed by 300 m^3 ha^{-1} of upper irrigation. Any alteration from this protocol results in insufficient control and tomato yield reduction. A solution to this problem may be elaboration of tomato variety resistant to imidazolinones herbicides. For this purpose EMS (ethyl methane sulfonate) mutagenesis was conducted on commercial tomato line M82. About 100.000 tomato seedlings (M2) were screened for resistance to pulsar (Imazamox). As a result, a novel tomato mutant HRT-1 was obtained. The mutant showed resistance to high rates to the imidazolinone herbicides Pulsar (Imazamox), Cadre (Imazapic), Arsenal (Imazapyr), and Staple (Pyrithiobac-sodium) from additional group of inhibiting acetolactate synthase herbicides (Pyrimidinylthiobenzoic acid). Application of these herbicides for broomrape control on HRT-1 and commercial tomato lines were tested. The results and the possible resistant mechanism will be discussed.

GENETICS OF *STRIGA* RESISTANCE IN EARLY AND EXTRA-EARLY MAIZE POPULATIONS ADAPTED TO THE SAVANNA ECOLOGY OF WEST AFRICA

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Maize (*Zea mays* L.) is a major staple food, feed and industrial crop in West Africa (WA). Maize grain production comes primarily from the Guinea savanna agro-ecology, which is characterized by a relatively short growing season thus necessitating the development and adoption of early and extra-early varieties. Maize production in the savanna is seriously constrained by several factors, which are beyond the control of resource-poor farmers who form the bulk of maize producers. One of the constraints is infestation by *Striga hermonthica* (Del.) Benth., a parasitic weed that has an intimate physiological relationship with the root system of the host plant. This relationship and the parasite's ability to produce large number of seeds that remain viable in the soil for 10 years or more limit the development of successful control measures within the reach of resource-poor farmers. Use of host-plant resistance is considered the most effective, economically feasible and sustainable means for *Striga* damage control. The International Institute of Tropical Agriculture (IITA), in collaboration with national agricultural researchers in WA, has developed maize populations and varieties with moderate resistance to *S. hermonthica*, two or which are early maturing, TZE-W Pop DT STR (white endosperm) and TZE-Y Pop DT STR (yellow) and two extra-early TZEE-W Pop DT STR and TZEE-Y Pop DT STR. The populations have been subjected to four cycles of S_1 recurrent selection and definite progress was made towards upgrading the *Striga* resistance and grain yield levels. Several studies were conducted to quantify the genetic variances, heritability estimates, genetic correlations and predicted progress from further selection. Three hundred full-sib families each were extracted from the C_3 of the early populations, TZE-W Pop DT STR and TZE-Y Pop DT STR and evaluated in separate studies. For the TZE-W Pop DT STR, additive genetic variances (σ_a^2) were positive, moderately large and much larger than the dominance variances (σ_a^2) for most traits. However, *Striga* emergence count was under the control of σ_d^2. Narrow sense heritability (h^2) was 25% for grain yield and 0-90% for 13 other traits. Grain yield had a large positive additive genetic correlation (r_a) with ears per plant (EPP), a large negative r_a with *Striga* damage ratings, and moderate negative r_a with flowering traits and *Striga* emergence count at 10 weeks after planting (WAP). For TZE-Y Pop DT STR, most traits had moderately large σ_a^2, although σ_d^2 was larger than σ_a^2 for grain yield and some other traits. Estimates of h2 were generally low for most traits (<0.40). In another study, 50 S1 families were each extracted from C0, C2, C3 and C4 of TZEE-W Pop STR and evaluated in *Striga* infested and *Striga*-free conditions. Gains in grain yield were 26% cycle^{-1} under *Striga* infestation and 16.4% ha^{-1} when *Striga*-free. Under *Striga*, genetic variances decreased with selection for *Striga* emergence and EPP. Under *Striga* free conditions, genetic variability also decreased for flowering traits. Genetic variances were significant for *Striga* emergence in all cycles and for EPP in C0 and C4. Response to selection for improved *Striga* emergence, EPP and grain yield are expected in subsequent cycles. Recurrent selection was effective for improving *Striga* resistance traits and grain yield which were characterized by low-to-medium heritability estimates.

BEHAVIOURS OF WINTER OILSEED RAPE FACING BROOMRAPE INFESTATION

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Broomrapes (*Orobanche* spp. and *Phelipanche* ssp.) are root parasitic plant devoid of chlorophyll that develop a haustorium serving as both an attachment organ to host roots and a bridge for water, mineral and organic nutrient uptake from host vascular tissues. Branched broomrape (*Phelipanche ramosa* (L.) Pomel, syn. *Orobanche ramosa* L. Pomel) is the most widespread broomrape in the world. This species causes severe damages to several crops in the Mediterranean region and southeast Europe, for a long time to hemp, tomato and tobacco, and more recently on winter oilseed rape (WOR). WOR is the primary European oilseed crop, and production areas have increased steadily since 2005, especially in France, Germany and in the eastern countries. In addition to known virulent oilseed rape pathogens such as insect and fungi, branched broomrape (genetic type 1, pathovar C) has become a major phytosanitary problem in WOR fields in the west part of France, causing heavy seed yield losses. Several control strategies are employed against broomrapes but none has enjoyed unequivocal success. The more economic and durable methods seem to be based in developing new varieties carrying several mechanisms of avoidance and resistance against *P. ramosa*. WOR is a recent host plant of the parasitic weed and such mechanisms are not characterized to date. We have initiated the identification of avoidance and resistance mechanisms among sixteen WOR cultivars which display different behaviours facing broomrape infestations in fields (CETIOM data). In order to access to a maximum of parasitism indexes, the interaction WOR – *P. ramosa* were performed in different conditions, including pot experiments in greenhouse and hydroponic cultures in growth chambers. A genetic variability occurs in the breeding materials, as indicated by the strong variability in the parasite incidence (number of attached broomrape per host plant) observed among the genotypes screened in greenhouse conditions. Hydroponic host-parasite co-cultures have shown that parasite incidence is correlated with the rate of broomrape seed germination in the vicinity to host roots in most of the varieties studied. Nevertheless some of them displayed some resistance to broomrape attachment following germination. A third behaviour was identified in WOR from both pot and hydroponic experiments, resulting in lower kinetics of attached broomrape growth and emergence from the soil (see abstract from Z. Gaudin et al.). These results are promising for the development of a durable resistance to broomrape in WOR by cumulating several mechanisms of resistance in new lines. Nevertheless additional studies are necessary for a better understanding of the genetic and molecular bases of these behaviors. These studies are financially supported by funds of the French organisations CETIOM, ONIDOL and PROMOSOL.

DIFFERENTIAL SUSCEPTIBILITY OF PEPPER *(CAPSICUM* SPP.) TO *PHELIPANCHE AEGYPTIACA*

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The wide host range of the root holoparasite *Phelipanche aegyptiaca* includes many Solanaceae crop plants such as tomato and eggplant. Peppers (*Capsicum* spp.) are known to be less susceptible to this parasite but in recent years pepper growers have reported crop infestation and losses due to *P. aegyptiaca*. The aim of this study was to screen pepper genotypes for resistance to *P. aegyptiaca*, to characterize the resistance and select resistant genotypes for future use in *P. aegyptiaca*-contaminated fields. We screened a collection of 21 pepper genotypes grown in pots in the greenhouse. The number of emerging parasite inflorescences was recorded throughout the experiments and fresh weights of parasite shoots, pepper shoots and fruits were determined at the termination of the experiments. In order to elucidate if the differential susceptibility was due to differential *P. aegyptiaca* seed germination induction, selected pepper genotypes were grown on GFA paper in plastic bags. The screening experiments revealed a wide range of *P. aegyptiaca* susceptibility from two highly susceptible genotypes supporting 15-17 parasite spikes, through reduced parasitism in seven genotypes that supported 5-9 spikes, to good resistance in 10 genotypes supporting less than three spikes. In most cases the resistant host varieties supported few poorly developed parasite inflorescences, probably due to incompatibility between host and parasite. In the highly susceptible pepper genotypes, fruit yields were 38-50% lower compared to the non-inoculated controls while in resistant genotypes, no yield reduction was recorded. Plastic bag studies with selected genotypes elucidated that the resistance was not due to reduced *P. aegyptiaca* seed germination induction. Further studies are in progress to detect the mechanisms responsible for the resistance phenomena and examination of the resistance under field conditions.

EXPRESSION ANALYSIS OF DEFENSE-RELATED GENES OF *VICIA FABA* L. PARASITIZED BY *OROBANCHE CRENATA*

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Orobanche crenata Forsk (broomrape), a parasitic angiosperm, is the major constrain for *Vicia faba* L. cultivation along Mediterranean basin. The broomrape attaches to the roots of faba bean, causing complete yield losses in the crops. Because no complete control measures are available, the genetic resistance of the host against the parasite remains as one of the most desirable components and environmental friendly methods within an integrated control strategy. But to date, the only available resistant faba bean cultivars exhibit an incomplete resistance. A detailed knowledge of the mechanisms underlying such resistance is required to improve breeding programs. However, the mechanisms underlying resistance against broomrape in faba bean are not well understood. Gene expression studies would help in understanding the molecular basis for the resistance and the mechanisms governing the host-parasite interaction. In order to gain insight into the identification of genes involved in the resistance mechanisms, the expression pattern of 17 putative genes were studied in the parental line faba bean (Vf136) with incomplete resistance against *O. crenata*. The candidate host plant genes were selected, based in several published studies, by their involvement in metabolic pathways such as the phenylpropanoid (*PAL*), ethylene (*ACC*) and jasmonate (*AOC, LOX, OPDAR* and *SPK1A*) biosynthesis pathways, and defensive mechanisms activated during several plant-parasitic plant interactions, including different genes reported for defense responses (*PR3, PR4, DFL, PRX, GLS* and *BGL*) and resistance gene analogs (*RGA01, RGA06, RGA08, RGA09* and *RGA10*). Transcript profiling of candidate genes during early stages of the faba bean/broomrape interaction (1, 3 and 7 d.a.i.) were carried out using quantitative real-time polymerase chain reaction (qPCR). The preliminary results indicate that genes from the jasmonate and ethylene pathways can be up-regulated in root tissues inoculated by broomrape. This work describes a first transcriptional approach with the aim of studying gene expression patterns in *V. faba* after infection with the parasitic plant *O. crenata*. Our results will contribute to elucidate the molecular basis of the resistance against this parasitic plant in *V. faba* and can be used as support for molecular or classic strategies of breeding for resistance.

GENOME WIDE SELECTION- MOVING BREEDING FOR *STRIGA* TOLERANCE INTO THE GENOMICS ERA

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At the International Institute to Tropical Agriculture maize breeders have for more than 4 decades worked towards the development of high yielding adapted *Striga* tolerant varieties. While heritability for *Striga* tolerance is moderate gain has been made using broad synthetics and recurrent selection. Many of the tolerance and resistance alleles used in the maize breeding work come from either west African landraces which have historically the longest co evolution time in the presence of *Striga* of any maize landraces in the world and which have not relative to east and southern Africa, been replaces by more modern materials. In addition important sources of resistance and tolerance have come from *Zea diploperennis* introgressed materials. We with CIMMYT have working under the Drought Tolerant Maize for Africa initiative have been using genome wide selection in marker assisted breeding activities to enhance the rate of development of drought tolerant germplasm for Africa. Some of the populations we work on have elite parents which in addition to drought tolerance also have *Striga* tolerance. This has enabled breeding using genome wide selection (GWS) for both *Striga* and drought while also facilitating QTL studies for more academic purposes. This presentation details some of the steps, processes and initial results obtained through the GWS application and plans for adoption of genomic selection in the future.

COMBINING ABILITY OF MAIZE INBRED LINES RESISTANT TO *STRIGA HERMONTHICA* EVALUATED UNDER ARTIFICIAL *STRIGA* INFESTATION IN KENYA

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Maize is an important food source in Africa. Its yield and production has been on the decline, making Africa and specifically Kenya a net importer of the grain. The parasitic weed *Striga* affects maize on an estimated 20 million ha, making it a major cause of maize yield reduction from near world average of 4.2t/ha a few decades ago to the present 1.3t/ha. The objectives of the present study were to examine the combining ability of twenty inbred lines and possibly to identify single crosses which can be used to develop three way and double cross hybrids resistant to *Striga hermonthica*. These lines were sourced from the International Institute of Tropical agriculture (IITA) (8), Kenya Agricultural Research Institute (KARI) (9) and the International maize and wheat improvement center (CIMMYT) (3). The IITA and KARI lines were *Striga* resistant while the CIMMYT lines were well adapted lines. Fourteen inbred lines (females) were factorially mated using Design II with six males all of which were from the IITA group. The resulting 84 F_1s along with six commercial checks were evaluated in four separate trials for two rainy seasons during the 2010 long rains and short rains. The trials were planted on station both under artificial *Striga* infestation and *Striga* free environments at Kibos and Alupe KARI stations in Kenya. Artificial *Striga* infestation was carried out by preparing an inoculum by mixing 5kg of fine sand with 10g of *Striga* seeds, then 7g of the inoculum was applied in each hill using table spoon to give an estimate of about 3,000 viable seeds and the maize seed was placed on top of the inoculum and covered well with soil. Data were recorded as *Striga* counts from 6 weeks after planting at an interval of 2 weeks up to the 12th week after planting. *Striga* damage rating was recorded using a score of 1-9, (1= no damage; 9= totally damaged) and flowering dates, plant height, ear height, and yield were also measured. General combining ability (GCA) and Specific combining ability SCA effects were calculated using line x tester analysis using SAS, where the females were considered as the lines and the males as the testers. GCA mean squares due to lines and testers were highly significant (P=0.001) for all traits. The ratio of GCA/SCA mean squares exhibited a predominance of additive gene effects in the inheritance of *Striga* resistance traits as opposed to dominant gene effects. Estimates of GCA effects indicated that six inbred lines were good combiners for grain yield. A number of single crosses out yielded all the six hybrid checks in both environments. These inbred lines may provide a reliable basis for further evaluation and even development of three way cross and double cross hybrids which can be grown in *Striga* prone areas thereby resulting in maize yield increment.

CAN WE USE ARBUSCULAR MYCORRHIZAL FUNGI TO IMPROVE RESISTANCE TO *OROBANCHE CUMANA* IN SUNFLOWER?

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Arbuscular mycorrhizal (AM) symbiosis is a mutualistic interaction between soil fungi and the roots of terrestrial plants. It is the most common symbiosis in the plant kingdom, and concerns over 80% of land plants. AM fungi are obligate biotrophs and rely on the photosynthetic carbon obtained from their host plants to complete their life cycle. In return, they supply their hosts with water and minerals, particularly phosphate. This association involves a molecular dialogue that allows mutual recognition of the two partners. AM fungi release compounds called Myc factors, and plant roots exude strigolactones, which stimulate fungal growth and metabolism and facilitate the first stages of AM symbiosis. Parasitic plants such as *Striga*, *Orobanche* and *Phelipanche* spp. use molecules released by plant roots as signals that trigger seed germination. This ensures that germination occurs in the vicinity of a host root, which is essential for parasitic seedling survival. Various metabolites induce parasitic seed germination including strigolactones, sesquiterpene lactones and dihydroquinones. Previous studies have shown that AM colonization of sorghum roots could decrease the level of Striga attachment and emergence. This effect appears to be due to the reduced ability of mycorrhizal root exudates to induce Striga seed germination. These observations have led to the hypothesis that mycorrhizal symbiosis could negatively regulate the production of strigolactones. The aim of our study was to determine whether a similar crop protection strategy could be used in the sunflower/*Orobanche cumana* interaction, which causes severe yield losses in Europe. It has to be noted that *O.cumana* is a specific parasite of sunflower. It was recently suggested that this specificity could be due to the release of a particular germination stimulant, dehydrocostuslactone, by sunflower roots. First, we observed that exudates from mycorrhizal sunflower roots induced less germination of *O. cumana* seeds than non-mycorrhizal root exudates. Thus, either mycorrhizal root exudates produce less germination stimulants, or they contain germination inhibitors. Biochemical analyses of strigolactone and dehydrocostuslactone contents are underway to address the first possibility. In addition, we tried to complement mycorrhizal root exudates with a synthetic strigolactone (GR24). This treatment was not sufficient to restore a germination rate comparable to that observed with non-mycorrhizal root exudates, or with GR24 alone. These observations suggest that mycorrhizal sunflower roots could produce inhibitors of *O.cumana* seed germination. We are currently investigating the physico-chemical properties of these putative inhibitors, and the biological conditions of their production.

STUDYING THE RESISTANCE OF CHICKPEA (*CICER ARIENTINUM*) GENOTYPES TO FIELD DODDER (*CUSCUTA CAMPESTRIS*)

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Field dodder (*Cuscuta campestris*) is an obligate stem holoparasite that parasitises various plant species worldwide, including crop plants such as chickpea, alfalfa, tomato and carrots and substantially reduces their yields. In recent years infestations are rapidly spreading, while control measures are insufficient. In screening studies conducted in a controlled environment greenhouse and in a net-house we have detected differential responses of chickpea genotypes to field dodder including two genotypes exhibiting consistent resistance to the parasite. The aim of this research is to elucidate the mechanisms of chickpea resistance to field dodder utilizing anatomic sectioning and microscopic examination. Samples of infected and uninfected plants were embedded in paraffin wax following fixation, dehydration and staining with safarnin that colors red the woody parts of the plant like lignin, suberin and cutin. The results show that in the resistant genotypes, host cortical and epidermal cells surrounding the haustoria were damaged, and that the haustoria failed to reach the host vascular tissues. We suggest that the resistant phenomenon may be attributed to compounds secreted by the host in the host-parasite interface. Future work will be aimed at detecting the specific stage and tissue involved in the resistance, and isolating the compounds responsible for the observed impeding of the invading parasite.

EFFECT OF DIFFERENT WATERLOGGING TREATMENTS ON *OROBANCHE FOETIDA* POIR. INFECTION ON FOUR FABA BEAN GENOTYPES

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Orobanche foetida Poiret is a serious threat to faba bean crops in Tunisia. In infested fields it causes major losses in production. Since there is no effective method of controlling the parasite, the development of resistant varieties remains the most economic and efficient way to solve this problem. Because the resistance is incomplete, using other means, such as waterlogging treatments, can help in reducing the infestation of the crop. In Tunisia, several *O. foetida*-infested fields are subject to a transient waterlogging which could be exploited to reduce *O. foetida* susceptible crops. A pot trial was carried out to study the effect of different waterlogging treatments (0, 3, 7, 12 and 21 days) on *O. foetida* infection of four faba bean small seeded genotypes. These comprised cv. Badï (susceptible to *O. foetida*) and cvs. Najeh, Baraca and XBJ90.04.6.2.1.1.4C (partially resistant to *O. foetida*). Faba bean genotypes were sown in the third week of December with four replicates with two plants per pot. Waterlogging treatments were applied at the beginning of faba bean flowering. *Orobanche* emergence date (OED), non emerged (NEO/P), emerged (EO/P), total (TO/P) tubercle numbers and fresh weights (EOFW/P, NEOFW/P, TOFW/P) per faba bean plant were recorded in the first week of May. Results showed a highly significant effect ($p<0.001$) of waterlogging on all recorded parameters. Compared to the control treatment, the different waterlogging treatments delayed the emergence of *Orobanche* on all genotypes except XBJ90.04.6.2.1.1.4C. For the 21 day waterlogging treatment the delay in *Orobanche* emergence was 8.5, 14.5 and 18.5 days for cv. Najeh, cv. Baraca and cv. Badï, respectively. This delay was proportional to waterlogging duration. Also, compared to the control, waterlogging decreased the level of infection, expressed on total *Orobanche* tubercule numbers per faba bean plant (TON/P). The decrease was 29%, 69%, 37% and 71% for cv. Badï, cv. Baraca, cv. Najeh and XBJ90.04.6.2.1.1.4C, respectively. The emerged *Orobanche* numbers per faba bean plant (EON/P) were decreased tremendously mainly for cv. Badï and cv. Najeh with average decreases of 59% and 64%, respectively. However non-emerged *Orobanche* numbers per faba bean plant (NEON/P) recorded for the two cultivars showed increases of 31% for Badï and 18% for Najeh. For all studied genotypes, the level of O. foetida infection decrease was accompanied by reductions of non emerged (NEOFW/P), emerged (EOFW/P) and total (TOFW/P) *Orobanche* fresh weight per faba bean plant. Average decreases of 28%, 49%, 57% and 72% were recorded in TOFW/P for cv. Badï, cv. Baraca, cv. Najeh and XBJ90.04.6.2.1.1.4C, respectively, and these decreases were observed mainly for EOFW/P.

CHARACTERIZATION OF THE GENETIC VARIATION IN RESISTANCE TO WITCHWEED (*STRIGA HERMONTHICA*) IN SORGHUM (*SORGHUM BICOLOR*) GERMPLASM COLLECTED FROM SUDAN

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In Sudan grain sorghum is the main food for most of the population. Sorghum production in the country is increasingly constrained by the parasitic weed *Striga hermonthica*. Development of resistant cultivars has been slow due to limited knowledge of the genetics of resistance to *Striga* and the difficulty in evaluating resistance in the field. Nonetheless many lines with a significant resistance level to *Striga* were identified among cultivated and wild sorghums. The aim of this research is to screen sorghum germplasm collected from Sudan, ICRISAT and Purdue University for genetic variation in quantitative and qualitative strigolactone production to identify *Striga* resistance mechanisms due to low germination stimulant activity. Sorghum varieties and land races commonly grown in Sudan in both the rainfed and irrigated areas are to be collected for evaluation of their reactions to *Striga* under laboratory, green house and field conditions. In vitro germination bioassays will be employed to rank sorghum genotypes based on their capacity to produce *Striga* germination stimulants. Ultra performance liquid chromatography / mass spectrometry is being utilized to measure strigolactone composition and concentration. Interesting germplasm will be further characterised using genetic and molecular approaches to determine the mechanism of the resistance. The study is expected to identify unique sources and levels of *Striga* resistance in the selected sorghum accessions. Research results are expected to verify potential resistant phenotypes characterized by low stimulant production. The research is expected to provide data about *Striga* resistance levels, potential modes of inheritance and the likelihood of stacking multiple resistance mechanisms (genes) in sorghum. Verified resistant/tolerant sorghum genotypes could be used as gene source for *Striga* resistance in sorghum improvement programs.

APPLICATION OF MOLECULAR MARKERS IN BREEDING COWPEA FOR RESISTANCE TO *STRIGA GESNERIOIDES*

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Cowpea, *Vigna unguiculate* (L.) Walp., is one of the most important food and forage legumes grown primarily in the semi-arid tropics. Yield losses due to parasitism by the root hemiparasitic weeds, *Striga gesnerioides* (Willd) [witchweed] to cowpea production in West and Central Africa are estimated in millions of tons annually and the prevalence of *Striga* soil infestation is steadily increasing. At least, seven races of *S. gesnerioides* have been identified within the cowpea growing regions of West Africa based on host differential response and genetic diversity analysis. The rapid spread of this parasitic weed creates an urgent need for cowpea variety with multiple resistance genes. Several race-specific resistance genes have been identified and located to one of two linkage groups (LG1 and LG2) of the current cowpea genetic map. Molecular markers have been identified that are associated with race-specific resistance genes, and several sequence-confirmed amplified regions (SCARs) have been developed for use in marker-assisted selection and breeding strategies for rapid cowpea improvement but no information is available on the efficiency of these markers. If the effectiveness of the new markers is validated, this should allow Marker-assisted Selection (MAS) to become more widely applicable in breeding cowpea for resistance to *S. gesnerioides*. This study was conducted to test the utility of three SCAR markers (MahSE2, E61R, and C42B) that have been developed for resistance to Striga race-3 in cowpea. An F_2 populations developed from the cross between Borno brown x IT03K-338-1 and Borno brown x IT97K-499-35 were evaluated to examine the genetic analysis of the populations segregating for *Striga* resistance against race 3 of *S. gesnerioides* and molecular characterization, to determine the efficiency of the different markers. Two of the marker systems MahSe2 and C42B showed high accuracy for discriminating between resistant and susceptible lines carrying SG3 resistance. The two markers also show DNA amplification consistency with high correlation to phenotypic score. These markers were not detected in lines carrying other genes other than the SG3. Genetic similarity index values among the two markers pairs varied from 0.45 to 0.95, with a mean of 0.98 for MahSe2 and 0.96 for C42B. The efficiency of these markers was further confirmed from the progeny testing of $F_{2:3}$ plants pulled from the F_3 seeds. This indicates the utility of these SCARs markers in MAS for SG3 race in cowpea breeding program.

BROOMRAPE (*OROBANCHE CUMANA* WALLR.) CONTROL BY BREEDING IN SUNFLOWER

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Broomrape (*Orobanche cumana* Wallr.) is a parasitic angiosperm that has been causing a great deal of damage to sunflower production for more than a century. There has been a constant tug-of-war between sunflower breeders and *Orobanche cumana*, with frequent changes in which side has the upper hand. Almost as soon as the breeders find a source of resistance to the latest race of the parasite, broomrape responds by evolving another virulent race. Sunflower selection for broomrape resistance makes use of different methods for testing breeding materials (in the field, greenhouse or at the molecular level), looks for resistance sources in certain wild species of genus *Helianthus* and has so far produced significant results. Dominant genes for resistance to races A, B, C, D, E and F have been found and incorporated into cultivated sunflower genotypes. In the last three years, new broomrape populations have been discovered in several countries (Turkey, Spain, Romania, Russia, Ukraine, Bulgaria). None of the existing commercial hybrids resistant to the race F has proven to be resistant to these new populations of the parasite. Greenhouse testing conducted at Fundulea institute in 2009 and 2010 years has managed to identify two restorer lines that are resistant to all the new populations. The resistance in these lines was found to be under the control of two dominant genes.

The rapid changes in broomrape race composition have forced sunflower breeders and geneticists to not only search for genes for resistance to new races of the parasite, but, to also look for alternative solutions to the problem of broomrape control.

Wild *Helianthus annuus* L. resistant to imidazolinones was first identified in Kansas, USA, in 1996. The use of imidazolinone resistance in sunflower breeding through the introduction of IMI-resistance into cultivated sunflower provides a broad spectrum of weed control and is specially effective in controlling *Orobanche* in sunflower. Using the sources IMISUN 1 and IMISUN 2 it has been obtained the first hybrids resistant to IMI herbicides, being cultivated in the CLEARFIELD system. But, in 2008, BASF company in collaboration with Nidera, Argentina, labeled the gene CLHA- Plus, the resistance being controlled by a partially dominant nuclear gene. The new gene is much more effective, giving the possibility to have a high level of the weed control, including *Orobanche*, in the new system CLEARFIELD Plus.

In the breeding program at Fundulea institute we are developing now the new sunflower lines, having the CLHA Plus gene.

FIELD EXPRESSION OF RESISTANCE AGAINST *STRIGA ASIATICA* AND *S. HERMONTHICA* IN THE NEW RICE FOR AFRICA (NERICA) CULTIVARS

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New Rice for Africa (NERICA) cultivars, a product of the crosses between the African rice species *Oryza glaberrima* (cv CG14) and the Asian *O. sativa* (*cv.* WAB56-104, -56-50 and -181-18), have become the flagship for boosting agricultural production in Africa with the aim to alleviate poverty and attain food security. Despite the reported wide distribution of these upland rice cultivars in *Striga* infested regions in Africa, little is known about their level of field resistance to *Striga* spp. Identification of resistant NERICA cultivars would be highly useful as the number of adapted rice cultivars with known resistance to any of the important *Striga* species (*S. asiatica* and *S. hermonthica*) is currently limited. Objectives of the current study were therefore to (1) broaden the pool of adapted upland rice germplasm with improved resistance against *Striga* spp. and (2) check the field expression of cultivars exhibiting pre- and post-attachment resistance mechanisms against these species as previously identified in the lab. To achieve these objectives 25 rice cultivars, including all 18 NERICA cultivars, their parents and local and susceptible checks, that have previously been evaluated for pre- and post-attachment resistance in labs of Wageningen University and the University of Sheffield respectively, have been tested for field resistance against *S. hermonthica* (in Mbita, Kenya, Lake Victoria region) and *S. asiatica* (in Kyela Tanzania, Lake Malawi region) in 2010. As the parents of the NERICA cultivars show different resistance mechanisms and levels, evaluation of resistance of their offspring will also provide us with valuable insights on the heritability of such traits. Similar to rankings based on pre- and post-attachment resistance from the labs, NERICA 2, 3, 5 and 10 (CG14 WAB56-104) and NERICA 17 (CG14 WAB181-18) showed high field resistance against both *S. asiatica* and *S. hermonthica*, while NERICA 7 (CG14 WAB56-104), 14 (CG14 WAB56-50) and 18 (CG14 WAB181-18) showed high susceptibility. The *O. glaberrima* parent CG14, being identified as resistant in the lab, was moderately susceptible against *S. asiatica* but resistant against *S. hermonthica*. The first field trial in Kyela, Tanzania with a population of *S. asiatica* that has co-existed with the same set of traditional cultivars (Kilombero and Mwangulu) for more than 30 years, showed significantly higher *Striga* infection levels on these traditional cultivars than on any of the other 23 introduced cultivars. This result generated three hypotheses: (1) local *Striga* ecotypes will be more adapted and hence more virulent to traditionally grown cultivars compared to newly introduced cultivars, (2) traditional cultivars, whilst more highly infected due to this *Striga* adaptation, will also show a higher degree of tolerance against the local *Striga* ecotype, compared to new cultivars, (3) genetic variation in resistance combined with differences in virulence between *Striga* individuals, ecotypes and species, determine *Striga* spp. infection levels in the field. Alongside confirmation of the expression of field resistance in the 25 cultivars evaluated in 2010, these hypotheses will be tested in the succeeding 2011 and 2012 seasons.

STUDIES ON PLANT ISOLATION AND HYBRIDISATION IN SUNFLOWER BROOMRAPE (*OROBANCHE CUMANA* WALLR.)

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Orobanche cumana Wallr. (sunflower broomrape) is a weedy parasitic angiosperm that represents a serious constraint for sunflower cultivation in many countries of Europe and the former USSR. Studies on sunflower resistance to *O. cumana* have concentrated so far on understanding the mechanisms underlying resistance in the host plant, but little is known about parasite virulence. Basic and methodological studies on *O. cumana* are required to gain insight into its breeding system and virulence genetics to define long term control strategies involving the complete host-parasite system. Accordingly, a methodological study was conducted to determine the feasibility of bagging *O. cumana* plants to force inbreeding and to produce F_1 hybrids under different bagging conditions. Seed production and seed quality in *O. cumana* was determined using different bag types to isolate flowering plants. The results indicated that *O. cumana* is self-compatible and tolerates seed production under isolation, both under self-pollination as well as in controlled hybridization. However, the bag type used for isolation clearly determined seed production and quality. Best results were obtained for plants isolated with micro-perforated transparent plastic bags that produced seeds that did not differ in germination and infectivity as compared to control unbagged plants. This bagging system was used to isolate an unpigmented mutant line from a single unpigmented plant detected in an *O. cumana* population from Central Spain. Hybridisation and isolation techniques were also used for the development of populations segregating for the trait. This study revealed that plant pigmentation in *O. cumana* was controlled by partially dominant alleles at a single locus. The feasibility of using isolation and hybridization techniques in *O. cumana* will facilitate basic studies on the reproductive biology of sunflower broomrape and the mode of inheritance of traits related to parasitism, which will contribute to the development of more stable sources of resistance to this parasitic weed.

Session 8

INTERACTIONS BETWEEN PARASITIC PLANTS AND THE ENVIRONMENT

Chair

Hanan EIZENBERG and Ahmet ULUDAG

INFLUENCE OF FERTILIZATION AND FIELD HISTORY ON SOIL PROPERTIES AND MICROBIAL COMMUNITIES AND ITS' RELATION TO *STRIGA HERMONTHICA* (DEL.) BENTH. POPULATION DENSITY, IN THE KATI DISTRICT OF MALI

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The parasitic plant *Striga hermonthica* (Del.) Benth. causes enormous yield losses particularly in cereal crops in the semi arid tropics, mainly in Sub-Saharan Africa. Beneath several control methods organic fertilization has controlling effects on *Striga*. Sauerborn et al. (2003) observed under the conditions of Ghana that the higher the organic matter content of the soil the lower is the *Striga* seed bank density and the less *Striga* emerged. The present research study investigates the relationship between field history, fertilization and *Striga* presence in the region around Sindala in Mali. Based on interviews with selected farmers, fields with high and low organic fertilization and different emergence of *Striga* were chosen for soil sampling and analysis. Soil was analyzed for phospholipid fatty acids (PLFA), dissolved organic matter (DOC), microbial C, N, and P and further relevant parameters like pH, texture, N, P, K, CEC, C_{org}. The results are interpreted with regard to the differences in soil microbial communities between high and low organic matter content and its impact on *Striga* emergence and seed bank density. This should lead to a more detailed knowledge whether organisms and/or other factors within the organic matter or facts relating to field history are playing a significant role in controlling *Striga* under a high organic fertilization practice.

DEVELOPING A PREDICTIVE MODEL BASED ON TEMPERATURES FOR *PHELIPANCHE AEGYPTIACA* PARASITISM IN CARROTS

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Egyptian Broomrape (*Phelipanche aegyptiaca*) is a root holoparasite plant that attaches to other plant roots. This plant is a severe threat in agriculture environment in the Mediterranean area in many field crops and vegetables, mostly from the Solanaceae, Fabaceae and Apiaceae. Carrot is grown in Israel between mid July (summer), and harvested until June (late spring) the next year. Depends on the level of infestation the potential damage in heavily infested field may reach to total yield loss. As a root parasite, that can be effectively controlled only in the soil subsurface, the prediction of the parasitism dynamic in these phase is a key stage in the development of a smart decision support system for *P. aegyptiaca* control. Therefore, the objective of the current study is to develop a robust prediction model for subsurface *P. aegyptiaca* parasitism in carrot. Ten field and controlled conditions experiments were conducted in Israel during 2010 and 2011, under various geographical and climatic conditions. The experiments employed a minirhyzotron camera, which allow non destructive in-situ subsurface observations of the parasite development. At each location, four transparent tubes artificially inoculated with *P. aegyptiaca* seeds, were buried in soil. Temperature of the top 10 cm from the soil surface was recorded. Observations for carrot root growth and *P. aegyptiaca* development were conducted once a week throughout the growing season. When carrot was sown in October, at soil temperature range of 22-25°C, broomrape attachments at size of 1-2 mm, were observed 30±2 days after sowing (DAS). However, in the late and cold December sowings, when temperature range was 16-17°C, attachments at size of 1-2 mm were observed after 47±2 DAS. When carrot was sown in late summer, there was a decline in broomrape development, and the appearance of 1-2 mm attachments appeared 42 DAS, significantly slower from the faster appearance of October although the average ambient temperature is higher (28°C). In the next stage of the study we will focus on the development and validation of a prediction model for *P. aegyptiaca* development in carrot based on the temperature data.

THE INFLUENCE OF ABIOTIC FACTORS ON *PHELIPANCHE* SPECIES INFESTATION IN TOBACCO FIELDS IN GREECE

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Abiotic parameters constitute the key factors that influence the interaction between host plants and broomrapes. The identification and quantification of the relationship between environmental variables and the infestation level of *Phelipanche aegyptiaca* and *Phelipanche ramosa* was examined for 144 tobacco fields for two consecutive years 2003-2004 in Greece. The fields were crossed in a zigzag line and four sampling points were inspected in each field across the line. The geographic coordinates of each sampling point were specified. Broomrape infestation was recorded by counting the number of parasites/crop plant within a radius of 60 cm. Soil samples were taken from 0-10 depth for each selected point and were analyzed in the laboratory for pH, organic matter content and the percentage of clay, silt and sand. Daily temperature and precipitation values were obtained from local meteorological stations for all study areas. These climatic variables were used in order to construct two bioclimatic parameters degree-days and Annual Humidity Index. All parameters were stored in a G.I.S. database which was developed for the purposes of this study. The determination of the most significant variables among the system soil-climate that influenced the infestation level by *Phelipanche* spp. on tobacco plants, was detected through a series of statistical analyses. The methods used included descriptive statistics (minimum, maximum and mean values, standard deviation), one way analysis of variance, Pearson correlation analysis, multiple regression analysis and discriminant analysis. Mapping the distribution of *Phelipanche* species gave a clear image of the variation in the infestation level among species, cultivated varieties and sampling areas. ANOVA showed significant differentiations among regions for all abiotic parameters. Pearson Correlation analysis demonstrated that the level of infestation was negatively correlated, statistically significant, with pH, AHI and positively with OM. Multiple regression analysis suggested that soil and climatic variables together explained 63% of the infestation variance. In discriminant analysis the first two functions accounted for 90% of the total variation in the dataset. All statistical analyses demonstrated that pH, AHI and OM were the most decisive variables to define infestation severity. The findings provide constructive information for modelling the broomrapes infestation level with abiotic factors and create a baseline for future monitoring the broomrapes distribution.

PREPARING AFRICAN RICE FARMERS AGAINST PARASITIC WEEDS IN A CHANGING ENVIRONMENT. A NEW, INTEGRATED RESEARCH PROJECT

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Rice is of strategic importance for attaining food security in sub-Saharan Africa. Parasitic weeds are progressively spreading in rain-fed rice production systems in the region due to their invasive nature, characterized by high reproduction rates and adaptive abilities, coupled with increased rice production areas and intensities. This is particularly worrisome considering projected climate changes. The most important parasitic weeds in rice are *Striga hermonthica*, *S. asiatica* and *Rhamphicarpa fistulosa*. Stakeholder analyses in three affected areas in West and East Africa showed that the problem is mainly affecting resource-poor farmers of which the majority is female. Farmers generally lack the knowledge and means to effectively control parasitic weed infestations. Extension services are not always aware of the actual extent of the problem and are often unable to backstop farmers with adequate solutions. This proved particularly true for *Rhamphicarpa*, which is a relatively new parasitic weed. The stakeholder analyses further showed the existence of a large time gap between the emergence of a parasitic weed problem and the start of any targeted action to control the problem. Clearly, improved communication between stakeholders and a better preparedness at farm, community and government level would help to reduce the extent and impacts of any future pest outbreak. Through a new, collaborative project of Wageningen University, Africa Rice Center and NARS of Benin, Côte d'Ivoire and Tanzania, funded through the Integrated Programme scheme of The Netherlands Organisation for Scientific Research, Science for Global Development (NWO-WOTRO), we aim to prepare the rice sector in threatened areas against projected increases in infestation levels .This will be done by investigating vulnerable locations, effects of climate variability and extremes on parasitic-weed survival, reproduction and virulence, economic impacts, and sustainable management strategies for resource-poor farmers. In addition, we will analyse how national crop protection systems function and innovate, taking parasitic weeds in rice-based cropping systems as a case study. Such analyses aid the development of policy guidelines geared to improve overall preparedness for new or increasing biotic constraints, as well as communication between different stakeholders. Interviews, chain analyses, field observations and measurements, yield-loss assessments and farmer-participatory experiments in hot-spots combined with controlled climate chamber experiments and different modelling approaches will enable us to develop meaningful scenarios of future invasions and agronomic and economic impacts, and develop feasible management and policy strategies for prevention and containment to support stakeholder decision-making.

CHARACTERIZATION OF SPATIAL PATTERNS OF *ORABANCHE AEGYPTIACA* IN COMMERCIAL TOMATO FIELDS IN ISRAEL

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Phelipanche aegyptiaca is a chlorophyll lacking root parasitic plant that has a broad host range. The damage from *P. aegyptiaca* is expressed in significant decrease in yield of the host plant and it can also cause host death. P. aegyptiaca is one of the main threats to tomato production in Israel. Many tomato fields are eventually abandoned by farmers as a result of heavy infestation with P. aegyptiaca. Knowledge about the spatial pattern of *P. aegyptiaca* in tomato fields and the dynamic of this pattern over time is essential in order to develop efficient control strategies. The objective of this study was to determine the spatial patterns within field of *P. aegyptiaca* and to examine the factors affecting this pattern. The rational is to develop a tool for mapping the parasitism levels in a time course, and specifically prior to tomato planning. Distribution of the *P. aegyptiaca* was determine in a regional and in plot scales. For regional scale, *P. aegyptiaca* infestation levels in tomato was collected from farmers through a survey in 2010. Additional data included information about crop rotation, history of nearby fields, containers location and herbicides treatments. In order to determine the pattern in the plot scale, *P. aegyptiaca* level was mapped in 15 tomato fields in 2010. The mapping was made using a GPS-GIS system. Every sample point represented a square of 240 m^2 [40 samples /hectare]. Every sample point was classified for a parasitism level based on a 0-3 index [0 - no parasitism; 3 - severe parasitism] based on shoot emergence. The sample maps were interpolated using Kriging to explore spatial patterns and apparent factors. Four spatial patterns were observed in the 15 plots and associated with apparent factors using the data collected for each plot through the regional survey: 1) Small clusters (Hot-spots) which were apparently caused from a specific infestation center (like container location or combine washing place); 2) Elongated clusters lengthwise to crop row which were apparently caused from agricultural equipment that was used in the plot; 3) Directional clusters which were apparently caused from the vicinity to a recently infected plot. In addition, an initial association was found with wind regime in the area: -when the neighboring infected plot was with the wind direction the directional cluster of the *P. aegyptiaca* was larger than when the neighboring plot was against the wind direction; 4) Randomly infected pattern which may characterize an established population of the P.aegyptiaca. After one season it is assumed that the agricultural activity has a considerable contribution to the spreading of the *P. aegyptiaca*. This research will continue for two more production seasons (2011-2012). Data will be collected in the regional and the plot scales. The multi-year database will enable a comprehensive analysis of the spatial patterns of the infection in the field and of the possible influencing factors. Such analysis will lead to an optimized sampling of the *P. aegyptiaca* presence in the field and will assist with a rationale management.

AN APPROACH CITIZEN SCIENCE TO MONITOR PARASITIC PLANT INVASIONS

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Citizen science is public involvement in projects or ongoing programmes of scientific work by which individual volunteers or networks of volunteers, many of whom may have no specific scientific training, perform or manage research-related tasks such as observation, measurement or computation. Improvements in internet and communication technologies lead involvement of citizens in monitoring and surveillance activities. The European Environment Agency has initiated citizen science activities by introducing Eye on Earth (EoE) which is an exploratory web-based IT platform for user-friendly, two-way sharing of environmental data and other environmental information with the general public and the scientific community. Current applications of these activities are Airwatch and Waterwatch. There is also the intention to develop other environmental watch activities including invasive alien species watch (IASwatch), which will be started as a pilot project in 2011. The project aims monitoring and surveillance of invasive alien species, supporting policy activities in Europe and targets of Convention on Biological Diversity, and raising awareness in general public. A group of IAS experts has been determined criteria to choose species for IASwatch. However, no organism has been chosen. Some parasitic plants can be considered as IAS although scientists of parasitic plants have been more familiar weed and endangered species terms. Due to global change, introduction of new species and expansion of ranges invaded by parasitic plants are expected as well as other organisms. EoE might be a tool of early warning of parasitic plants, which is one of the main elements of IAS strategies. It is unclear to include a parasitic plant species at pilot phase of IASwatch, it could be included in broad-application phase.

UNDERSTANDING CLIMATE CHANGE ON PARASITIC PLANTS' INVASIONS

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Climate change and its effects are not understood enough in general in spite of existence of clear indicators which have been observed and projected. Adaptation to climate change which is required to lessen adverse effect of the climate changes and get benefit of some changes is an issue world wide in all sectors. Observations and predictions show changes in species-ecosystem relations, phenology, forestry and farming. There has been a little scientific work on effect of climate change on parasitic plants. North/upward shift of plant species is predicted including parasitic plants. Changes at host range and host preference might be an additional point to be considered predicting distribution of parasitic plants. Elevating temperature and CO2 will effect host-parasitic relation and farming practices, which will also effect indirectly host-parasite relation and shifting. All strategies used on invasive alien species could be applied for parasitic plants too. However, models showing climate change effects on parasitic plants and their relations with the environment are needed to understand the issue well and plan future policies and scientific activities.

MALVACEAE WEEDS AS HOSTS FOR *PHELIPANCHE AEGYPTIACA* IN ISRAEL

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Broomrape parasitism has a harmful effect on the growth and yield of several economically important host crops. Egyptian broomrape (*Orobanche aegyptiaca* Pers. = *Phelipanche aegyptiaca*) is an obligatory haloparasite that attacks the roots of many dicotyledonous crops and weeds. This is the most dangerous parasite of all broomrape species parasitizing watermelons, melons, cucumbers, tomatoes, eggplants, potatoes, tobacco, peppers, cabbage, carrots and sunflowers. Weeds belonging to the Malvaceae such as *Malva* spp., *Lavatera* spp. *Abutilon theophrasti* and *Hibiscus* spp. are common in Israel and other Mediterranean countries, in crops such as wheat, cotton, sunflower, corn and cucurbits. During the growing season of summer 2008 we found a population of *A. theophrasti*, attacked by *P. aegyptiaca* in Ein Dor in the Jezreel valley. This field was heavily infested by broomrape seeds following infestation of host crops in previous years. At a nearby location, another Malvaceae weed e.g; *Malva nicaeensis*, was severely attacked by *P. aegyptiaca* in an olive orchard (*Olea europea*) which was previously a tomato field. Excavation of the parasite and host roots clearly verified connections between the parasite and the *Abutilon* or *Malva* roots. Seeds of Egyptian broomrape collected from a highly infested field in Gesher Haziv in the Western Galilee effectively parasitized *A. theophrasti* population when grown in pots in the greenhouse in the summer of 2009. These results are representing only preliminary data but show that some biotypes of *P. aegyptiaca* attack tomato and Malvaceae plants with equal vitality. To the best of our knowledge, this is the first time that a Malvaceae species in general and *Abutilon* spp. or *Malva* spp. in particular has been reported to serve as host for *P. aegyptiaca*. Due to heavy infestation with Egyptian broomrape in agricultural fields in the Mediterranean, we assume that there are many different parasite populations, and that the development of violent biotypes can increase the range of crops becoming potential hosts to Egyptian broomrape. It was observed in recent years that violent sunflower broomrape biotypes from the Hula Valley effectively attacked resistant sunflower varieties in other areas of the country, indicating a continuous evolution of new strains of the parasite and the need for more research in this area.

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