



12th World Congress on Parasitic Plants

15th-20th July 2013 - Sheffield, UK

International Parasitic Plant Society

12th World Congress on Parasitic Plants

15th – 20th July, Sheffield, United Kingdom.

PROGRAMME AND ABSTRACTS

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FOREWARD

Welcome to the 12th World Congress on Parasitic Plants. We are delighted to welcome everyone to Sheffield where there is a long tradition of parasitic plant research.

We are looking forward to meeting researchers from all over the world for an exciting and varied scientific programme. As well as excellent science the meeting will provide an unparalleled opportunity for networking and discussion as well a range of diverse social events including a visit to the world-renowned Peak District National Park.

The Congress will bring 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 will include presentations at the cutting edge of parasitic plant research and of management technologies of parasitic weeds. We are looking forward to meeting you at the Congress.

The congress' official website is:

http://ipps13.group.shef.ac.uk/index.html

The website will remain active after the congress and will contain details of abstracts and photographs from the event.

Julie Scholes, Duncan Cameron and Koichi Yoneyama

ACKNOWLEDGEMENTS

We are very grateful to Syngenta for their sponsorship of the congress and to the International Institute of Tropical Agriculture (IITA) for their generous financial support for African Scientists. We also thank the University of Sheffield for hosting the meeting and Linda Dully (Animal and Plant Sciences), Lisa Knight and Kelly Gibson (Finance Department) for invaluable administrative support.

Many thanks for the excellent efforts of the session organizers and wider organising committee who selected and put together an exciting programme and have reviewed all of the abstracts.

Julie Scholes and Duncan Cameron

ORGANISING COMMITTEE

Programme Chair and President of the International Parasitic Plant Society:
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WCPP[12] Programme Committee:

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Philippe DELAVAULT	France
Julie SCHOLES	UK
Ahmet ULUDAG	Turkey
John YODER	USA

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

PROGRAMME AT A GLANCE

Sunday 14th July

14.00 – 21.00 Arrival and Registration

Monday 15th July

09.00 – 20.00 Arrival, Registration and Poster setup

19.30 – 22.30 *Welcome Reception and Opening of WCPP*[12]

Tuesday 16th July

08.30 – 11.00 **Strigolactones: Structure and Function**

10.30 – 11.00 *Coffee break*

11.00 – 13.30 **Strigolactones: Structure and Function (continued)**

12.30 – 13.30 *Lunch*

13.30 – 15.30 **Session 1: Genomics**

15.30 – 16.00 *Tea break*

16.00 – 16.30 **Session 1: Genomics (continued)**

16.30 – 18.05 **Session 2: Biology-Biochemistry Part 1**

19.30 – 22.30 ***Yorkshire themed evening meal***

Wednesday 17th July

09.00 – 09.45 **Session 2: Biology-Biochemistry Part 2**

09.45 - 10.35 **Session 3: Ecology and Population Biology**

10.35 – 11.05 *Coffee break*

11.05 – 12.00 **Session 3: Ecology and Population Biology**

12.00 – 21.00 ***Picnic Lunch, Excursion to Chatsworth House and BBQ***

Thursday 18th July

09.00 – 10.25 **Session 4: Control and Management**

10.25 – 11.00 *Coffee break*

11.00 – 12.00 **Session 4: Control and Management**

12.00 – 13.30 *Lunch*

13.30 – 15.25 **Poster Session, Discussion and Tea Break**

15.25 – 17.20 **Session 5: Crop Resistance and Tolerance**

19.30 – 22.30 ***Conference Dinner***

Friday 19th July

09.00 – 10.30 **Session 6: Environmental factors, modeling and mapping**

10.30 – 11.00 *Coffee break*

11.00 – 11.50 **Session 6: Environmental factors, modeling and mapping**

11.50 – 12.25 **Session 7: Host-Parasite Communication**

12.25 – 14.00 *Lunch*

14.00 – 16.00 **Session 7: Host-Parasite Communication**

16.00 – 16.30 *Tea break*

16.30 – 18.00 *General Discussion / Poster & Student Prize*

19.30 – 22.30 *Lamb Roast on the Piazza and Disco*

Saturday 20th July - Departures

DETAILED PROGRAMME

Sunday 14th July

All day Arrival

(Accommodation at the Edge available from 14.00 h)

Monday 15th July

All day Arrival

09.00 – 20.00 Registration and Poster setup

19.30 – 22.30 *Welcome Reception and Opening of WCPP*[12]

Tuesday 16th July

Strigolactones: Structure and Function

08.30 – 08.40 *Welcome by Cristina Prandi and Hinanit Koltai*

08.40 – 09.20 **Keynote lecture: Binne Zwanenburg**

Advances and challenges in strigolactone research.

09.20 – 09.50 **Keynote lecture: Salim Al-Babili**

Strigolactone biosynthesis: Few enzymes for a complex backbone.

09.50 – 10.10 **Kotomi Ueno**

The bioconversion of 5-deoxystrigol to mono- hydroxylated strigolactone by plants.

10.10 – 10.30 **Carolien Ruyter-Spira**

Natural variation in strigolactone biosynthesis in rice is associated with structural variation and deletion of two *MAX1* orthologs.

10.30 – 11.00 *Coffee break*

11.00 – 11.20 **Keynote lecture: Yoram Kapulnik**

Biological and functional activity of different strigolactone analogues.

11.20 – 11.35 **Evgenia Dor**

Characterization of new tomato varieties lacking strigolactones.

11.35 – 11.50 **Takahito Nomura**

The effects of phosphate and nitrogen nutrients on the production of strigolactones in Arabidopsis.

11.50 – 12.10 **Kosuke Fukui**

Debranones partially and selectively mimic strigolactone function.

12.10 – 12.30 **David C. Nelson**

An investigation of the genetic basis for strigolactone perception in parasitic plant germination.

12.30 – 13.30 *Lunch*

Session 1: Genomics

13.30 – 13.35 *Welcome by John Yoder and Jim Westwood*

13.35 – 14.05 **Keynote lecture: Ken Shirasu**

Genome and transcriptome analyses of *Striga* spp.

14.05 – 14.35 **Keynote lecture: Claude W. dePamphilis**

Tissue specific *de nove* transcriptomics in the parasitic Orobanchaceae.

14.35 – 14.45 **Loren A. Honaas**

Genome scale analysis of laser micro-dissected tissues sheds light on parasitic plant-host plant interactions.

14.45 – 15.00 **Steven Runo**

Agrobacterium rhizogenes transformation of *Zea mays:* a functional genomics tool for host-parasite interaction.

15.00 – 15.15 **John Yoder**

Trans-specific gene silencing: a biological strategy to control parasitic weeds?

15.15 – 15.30 **Dina Plakhine**

Genetic aspects of stimulant specificity in *Orobanche* seed germination.

15.30 – 16.00 *Tea break*

16.00 – 16.15 **Gunjune Kim**

De novo transcriptome assembly of *Cuscuta pentagona* and bidirectional movement of mRNA between hosts and parasite using high-throughput sequencing.

16.15 – 16.30 **Jim Westwood**

Characterization of mobile RNA from hosts to *Cuscuta pentagona.*

Session 2: Biology-Biochemistry Part 1

16.30 – 16.35 *Welcome by Philippe Delavault and Philippe Simier*

16.35 – 16.55 **Marc-Marie Lechat**

CYP707A1, an ABA catabolic gene, is a ubiquitous component of parasitic plant seed germination in response to various germination stimulants.

16.55 – 17.15 **Takatoshi Wakabayashi**

Inhibitory effect of nojirimycin on germination and sugar metabolism of a broomrape.

17.15 – 17.35 **Tal Shilo**

Aspects of glyphosate mechanism in Egyptian broomrape control.

17.35 – 17.50 **Luiza Teixeira-Costa**

Anatomical and functional changes on the host wood caused by the infestation of *Phoradendron crassifolium* (Viscaceae).

17.50 – 18.05 **Juan A. Lopez-Raez**

Plant defence responses against root parasitic plants.

19.30 – 22.30 ***Yorkshire themed evening meal***

Wednesday 17th July

Session 2: Biology-Biochemistry Part 2:

09.00 – 09.15 **Anna J. Wiese**

The chemical nature of parasitic and mycoheterotrophic metabolism involves the reconfiguration of substrate usage in order to sustain the tricarboxylic acid cycle.

09.15 – 09.30 **Jason D. Smith**

Parasitic plants imbibe host plant toxins that influence insect herbivores.

09.30– 09.45 **Ai-Rong Li**

Nutrient requirements differ in two *Pedicularis* species in the absence of a host plant: implication for driving forces in the evolution of host preference of root hemiparasitic plants.

Session 3: Ecology and Population Biology

09.45 – 09.50 *Welcome by Yaakov Goldwasser and Jonne Rodenburg.*

09.50 – 10.05 **Gui-Lin Chen**

The distribution and evolution of the genus *Cynomorium.*

10.05 – 10.20 **Peter Tóth**

Broomrape pollinators in the light of floral volatiles.

10.20 – 10.35 **Nina Hobbhahn**

Pollination systems in *Cytnus*: ants, rodents, elephant shrews, and more.

10.35 – 11.05 *Coffee break*

11.05 – 11.20 **Iliya Denev**

A molecular taxonomy study on *Phelipanche* species (Orobanchaceae) in Bulgaria.

11.20 – 11.35 **Jane Prider**

Natural seed bank decline of *Phelipanche mutelii* in South Australia.

11.35 – 12.00 *Discussion*

12.00 – 21.00 ***Picnic Lunch, Excursion to Chatsworth House and BBQ***

Thursday 18th July

Session 4: Control and Management

09.00 – 09.05 *Welcome by Jonne Rodenburg and Yaakov Goldwasser*

09.05 – 09.25 **Jonne Rodenburg**

The potential of timing as a parasitic weed management strategy for smallholder rice farmers.

09.25 – 09.45 **Meva Tahiry Randrianjafizanaka**

The role of resistant rice varieties in a locally adapted integrated *Striga* management approach.

09.45 – 10.05 **Yaakov Goldwasser**

Phelipanche aegyptiaca control in tomato by application of imazapic through drip irrigation.

10.05 – 10.25 **Alistair J. Murdoch**

Effects of *Desmodium* root exudates on *Phelipanche ramosa* and *Orobanche crenata* and other associated hosts.

10.25 – 11.00 *Coffee break*

11.00 – 11.20 **Gregório Ceccantini**

Shoot the mistletoe - A new method for controlling mistletoes in trees.

11.20 – 11.40 **Djibril Yonli**

Use of potential non-host crop genotypes and allelopathy properties of local plants for controlling *Striga hermonthica* in Burkina Faso.

11.40 – 12.00 Discussion

12.00 – 13.30 *Lunch*

13.30 – 15.15 **Poster Session and Discussion**

Chairperson: Harro Bouwmeester

Tea break

Session 5: Crop Resistance and Tolerance

15.25 – 15.30 *Welcome by Mike Timko and Julie Scholes*

15.30 – 16.00 **Keynote lecture: Michael M. Timko**

Identification of genes controlling compatible and incompatible interactions of cowpea with *Striga. gesnerioides*.

16.00 – 16.20 **Boubacar A. Kountche**

Breeding for *Striga* resistance in pearl millet: response to five cycles of recurrent selection.

16.20 – 16.40 **Xi Cheng**

Natural variation in resistance against parasitic plants

16.40 – 17.00 **Oz Ben David**

Variation in response of a resistant sunflower cultivar to *Phelipanche aegyptiaca* and *Orobanche cumana*.

17.00 – 17.20 **Anne-Laure Hepp**

Metabolomic analysis of the resistance response in sunflower roots to the parasitic weed *Orobanche Cumana.*

19.30 – 22.30 ***Conference Dinner***

Friday 19th July

Session 6: Environmental factors, modeling and mapping

09.00 – 09.05 *Welcome by Hanan Eizenberg*

09.05 – 09.45 **Keynote lecture: Hanan Eizenberg**

Tempo-spatial modeling of broomrapes (*Orobanche* and *Phelipanche* spp.) parasitism - a key for their sustainable management.

09.45 – 10.15 **Abebe Menkir**

Combining resistance to *Striga hermonthica* with tolerance to drought in maize.

10.15 – 10.30 **Amnon Cochavi**

Development of a decision support system based on modeling approach for Egyptian broomrape (*Phelipanche aegyptiaca*) control in carrot.

10.30 – 11.00 *Coffee break*

11.00 – 11.15 **Gregório Ceccantini**

Using microtomography techniques to better understand the anatomical interface between host and parasite.

11.15 – 11.30 **Simon N'cho**

Factors affecting parasitic weed infestation in rain-fed lowland rice systems: the case of *Rhamphicarpa fistulosa* in Benin.

11.30 – 11.50 *Discussion*

Session 7: Host-Parasite Communication

11.50 – 11.55 *Welcome by Koichi Yoneyama and Maurizio Vurro*

11.55 – 12.25 **Keynote lecture: Harro Bouwmeester**

Regulation of parasitic plant germination.

12.25 – 14.00 *Lunch break*

14.00 – 14.15 **Kaori Yoneyama**

Difference in *Striga*-susceptibility correlates with 5-deoxystrigol exudation but not with compatibility/selectivity to AM fungi in maize.

14.15 – 14.30 **Johann Louam**

Can we use arbuscular mycorrhizal fungi to improve resistance to *Orobanche cumana* in sunflower?

14.30 – 15.00	**Keynote lecture: Daniel M. Joel**
	The haustorium of the Orobanchaceae – a review.
15.00 – 15.15	**Jeffery J. Morawetz**
	Comparative haustorial morphology and structure in parasitic Orobanchaceae.
15.15 – 15.30	**Takanori Wakatake**
	Dynamic changes in cell morphology during haustorium development in *Phtheirospermum japonicum*.
15.30 – 15.45	**Juliane K. Ishida**
	Functional identification of the genes involved in haustorium development in the facultative parasitic plant *Phtheirospermum japonicum.*
15.45 – 16.00	*Discussion*
16.00 – 16.30	*Tea break*
16.30 – 18.00	*General Discussion / Poster & Student Prize*
19.30 – 22.30	*Lamb Roast on the Piazza and Disco*

Saturday 20th July

All day	Departures

Poster presentations

Session 1: Genomics

Radoslava Matusova

Agrobacterium-mediated transformation of *Phelipanche ramose.*

Yasunori Ichihashi

Transcriptomics in parasite development of *Striga hermonthica*

Session 2: Biology and Biochemistry

Grégory Guirimand

Functional characterization of a β-mannosidase involved in the early germination process of *Orobanche minor.*

Thomas Péron

Characterization of the genes encoding sucrose transporters and sucrose-degrading enzymes in the parasitic plant *Phelipanche ramose.*

Philippe Simier

Genetic and phenotypic diversities in the parasitic species *Phelipanche ramose.*

Luiza Teixeira-Costa

Comparative phenology of two parasitic plants of the genus *Struthanthus* (Loranthaceae) infesting two different hosts.

Kristen Clermont

Metabolomic analysis of early stages of *Phelipanche aegyptiaca* development.

Session 3: Ecology and Population Biology

María Paz-Ponce

Report on *Ceroplastes* sp in mistletoe (*Phoradendron bolleanum*) Sierra de Arteagam Caohuila, Mexico.

Alpha Y. Kamara

Assessment of the level and extent of *Striga* infestation of cereal and cowpea fields in a dry savanna ecology of northern Nigeria.

Stella Kabiri

Ecological niche differences between *Rhamphicarpa fistulosa* and *Striga asiatica* in rain-fed rice.

Nina Hobbhahn

Limitation of current reproduction by resource availability and mating costs in two South African *Harveya* species - An experimental field study.

Session 4: Control and Management

Lum A. Fontem

Combating purple witchweed (*Striga hermonthica* (Del.) Benth.) with acetolactate synthase-modified maize seeds in the West African savannas.

Emmanuel I. Aigbokhan

Screening effects of crude aqueous sawdust extracts on germination of *Striga hermonthica* seeds.

Musa G. M. Kolo

Management of *Striga hermonthica* with *Aeschynomene histrix* in maize (*Zea mays* L.).

Daniel T. Gungula

Reactions of different genotypes of maize treated with varying rates of imazapyr in Yola Nigeria.

Rosemary Ahom

Studies on the potential of neem tree products as bioagents for management of *Striga hermonthica* in maize.

María Paz-Ponce

Isolation of fungi infecting mistletoe, *Phoradendron macrophyllum,* at Saltillo, Mexico.

Session 5: Crop Resistance and Tolerance

Baffour Badu-Apraku

Combining ability and heterotic patterns of quality protein maize inbreds under *Striga*-infested environments.

Hiroaki Samejima

Evaluation of resistance of upland rice varieties to *Striga hermonthica* through laboratory, pot and field experiments.

Lucky O. Omoigui

Identification of new sources of resistance to striga gesnerioides in cowpea accession

Dan Kiambi

Evaluation of marker assisted Breeding Striga resistant sorghum varieties in Eastern and Central Africa.

Session 6: Environmental Factors, Modeling and Mapping

No posters

Session 7: Host-Parasite Communication

Yukihiro Sugimoto

Structural requirements of strigolactones for germination induction and inhibition of *Striga gesnerioides* seeds.

Xiaonan Xie

Novel germination stimulants for root parasitic plants produced by *Nicotiana tabacum* L.

Takaya Kisugi

Identification of strigolactones produced by a Chinese medicinal plant *Houttuynia cordata.*

Hyun Il Kim

Novel strigolactones produced by black oat.

Yu-xia Song

Effects of exogenous substances on parasitism of *Cistanche deserticola.*

ABSTRACTS

Strigolactones: Structure and Function

Chairs

Cristina Prandi and Hinanit Koltai

ADVANCES AND CHALLENGES IN STRIGOLACTONE RESEARCH

Binne Zwanenburg

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Department of Organic Chemistry, The Netherlands

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Parasitic weeds have a devastating impact on the development of the host plants. When agriculturally important crops are involved, the food production may be severely affected, which often is the case in tropical and semi-tropical areas in Africa and Asia. The lifestyle of the root parasites is extremely well adapted to that of their host plants. Abundant evidence is available that strigolactones (SLs) are the true stimulants for germination of seeds of parasitic weeds. Over the years our research program focused on the chemistry and structure-activity relationship of germination stimulants for parasitic weeds. In this lecture recent advances in this area will be presented: new bioactive strigolactone analogues, new bioactivities of SLs, new SL mimics. Challenges are to understand the mode of action of SLs and how to apply SL analogues in the field to control parasitic weeds. As SLs are now considered as new plant hormones, it is relevant to compare structure activity relationship for various bio-activities and how suitable structurally simpler analogues can be designed and prepared for each of them. The main focus is providing insight in the molecular behavior of these new plant hormones.

STRIGOLACTONE BIOSYNTHESIS: FEW ENZYMES FOR A COMPLEX BACKBONE

Salim Al-Babili

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Besides their known role as photosynthetic pigments, carotenoids fulfill a more general function in animas, fungi and plants as a source of signaling molecules that arise by oxidative cleavage catalyzed by the ubiquitous family of carotenoid cleavage dioxygenases. Strigolactones are a recent example of carotenoid-derived phyto-hormones. Strigolactones have a complex structure consisting of a tricyclic lactone (A, B, and C rings) connected via an enol ether bridge to a second lactone (D-ring). It was supposed that the biosynthesis of SLs is a complex process requiring many enzymes that form the ABC part from carotenoids, which is then coupled - in a later step - to a lactone (D-ring) of unknown origin. However, recent investigation revealed that the pathway starts with a novel *cis*/*trans*-isomerase followed by two carotenoid cleavage dioxygenases the first of which is stereo-selective while the second one is an unusual enzyme that catalyzes a combination of reactions leading to carlactone, a novel compound that already shows typical structural features of strigolactones and exerts their biological activities. Carlactone will be crucial for understanding the biology of strigolactones and bears potential for different applications in agriculture.

THE BIOCONVERSION OF 5-DEOXYSTRIGOL TO MONOHYDROXYLATED STRIGOLACTONES BY PLANTS

Kotomi Ueno, Noriko Motonami, Hitomi Nakashima, Saki Nomura, Masaharu Mizutani, Hirosato Takikawa, Yukihiro Sugimoto

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Strigolactones, important rhizosphere signalling molecules and a class of phytohormones that control shoot architecture, are apocarotenoids of plant origin. They have a structural core consisting of a tricyclic lactone connected to a butyrolactone group via an enol ether bridge. Deuterium-labelled 5-deoxystrigol stereoisomers were administered to aquacultures of sorgomol-producing plants, sorghum (*Sorghum bicolor*) and Chinese milk vetch (*Astragalus sinicus*), and a strigol-producing plant, cotton (*Gossypium hirsutum*). Conversion of these substrates to sorgomol and strigol stereoisomers was investigated. Liquid chromatography-mass spectrometry analyses established that 5-deoxystrigol (5-DS) and *ent*-2'-*epi*-5-deoxystrigol were absorbed by sorghum roots, converted to sorgomol and *ent*-2'-*epi*-sorgomol, respectively, and exuded out of the roots. On the other hand, only 5-DS was converted to sorgomol and strigol by root of Chinese milk vetch and cotton, respectively. These conversions were inhibited by uniconazole-P, implying the involvement of cytochrome P450 in the hydroxylation. These results provide experimental evidence for the postulated biogenetic scheme for biosynthesis of strigolactones, in which hydroxylation at C-9 and C-5 of 5-DS can generate sorgomol and strigol, respectively.

NATURAL VARIATION IN STRIGOLACTONE BIOSYNTHESIS IN RICE IS ASSOCIATED WITH STRUCTURAL VARIATION AND DELETION OF TWO *MAX1* ORTHOLOGS

Catarina Cardoso[1], Yanxia Zhang[1], Muhammad Jamil[1], Jo Hepworth[2], Tatsiana Charnikhova[1], Stanley Dimkpa[3], Caroline Reif[4], Mark Wright[5], Susan McCouch[5], Yonghong Wang[6], Ottoline Leyser[7], Adam Price[3], Harro J. Bouwmeester[1,8], Carolien Ruyter-Spira[1,9]

[1] Laboratory of Plant Physiology, Wageningen University, the Netherlands; [2]Department of Biology, University of York, UK; [3] Institute of Biological and Environmental Sciences, University of Aberdeen, UK; [4] GenePool Hub Spoke Next-Generation Sequencing Bioinformatician, University of Aberdeen, UK; [5] Department of Plant Breeding & Genetics, Cornell University, USA; [6] State Key Laboratory of Plant Genomics and National Center for Plant Gene Research, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China; [7] Sainsbury Laboratory, University of Cambridge, UK; [8]Centre for Biosystems Genomics, Wageningen, the Netherlands; [9] Plant Research International, Bioscience, Wageningen, the Netherlands.

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Strigolactones are phytohormones regulating shoot branching, root architecture and secondary growth. However, strigolactones also act as signalling molecules in the rhizosphere. Here, they not only serve as host detection signals for arbuscular mycorrhizal (AM) fungi, but also stimulate germination of root parasitic plants. Interestingly, in rice low SL producing varieties display reduced *Striga* infection levels. A number of genes (*D27, CCD7, CCD8, MAX1*) have been shown to encode the strigolactone biosynthetic pathway. Strigolactone biosynthesis increases when plants experience phosphate starvation. To gain a better understanding of the regulation of strigolactone production in phosphate starved rice plants, we followed a genetic approach. An F_6 mapping population was used for which the parental lines (Bala x Azucena) showed a significant difference in the SL level in root extracts and exudates. SL analysis in this population, followed by mapping, resulted in the identification of a major QTL on chromosome 1 for the level of all five strigolactones that we could detect. Sequence analysis of the corresponding locus revealed a rearrangement of a 51-59 Kbp stretch between 28.9 and 29 Mbp in the Bala genome, resulting in the deletion of two cytochrome P450 genes highly similar to the *Arabidopsis* SL biosynthesis gene, *MAX1*. The expression of one of these P450s is, like SL biosynthesis itself, induced upon phosphate starvation and this P450 is therefore considered a strong candidate to be involved in the SL biosynthetic pathway.

BIOLOGICAL AND FUNCTIONAL ACTIVITY OF DIFFERENT STRIGOLACTONES ANALOGUES

Maja Cohen[1,2], Cristina Prandi[3], Ernesto G. Occhiato[4], Silvia Tabasso[3], Smadar Wininger[1], Nathalie Resnick[1], Yosef Steinberger[2], Hinanit Koltai[1], Yoram Kapulnik[1]

[1] Institute of Plant Sciences, ARO, Volcani Center, Bet Dagan, Israel

[2] The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan, Israel

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Strigolactones (SLs) have several functions as signaling and plant hormone molecules. Their interactions with symbiotic arbuscular mycorrhizal (AM) fungi, the parasitic weeds *Orobanche* and *Striga* and plant development of both root and shoot have been described. By comparing the activity of synthetic SL analogs on *Arabidopsis* root-hair elongation, *Orobanche aegyptiaca* seed germination, and hyphal branching of the AM fungus *Glomus intraradices*, we found that each of the tested organisms differs in its response to the various examined synthetic SL analogs. Structure–function relations of the SL analogs were studied. Description of competitive antagonistic analogs suggests that the A-ring of SL can affect not only affinity to the receptor, but also the molecule's ability to activate it. It was concluded that *Arabidopsis*, *Orobanche*, and AM fungi possess variations in receptor sensitivity to SL analogs, probably due to variation in SL receptors among the different species. Moreover, in this presentation a potential substitute to the ultimate SL analog, GR24, will be presented and the biological relevance in different experimental systems will be shown.

CHARACTERIZATION OF NEW TOMATO VARIETIES LACKING STRIGOLACTONES

Evgenia Dor, Evgeny Smirnov and Joseph Hershenhorn

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One of the most destructive pests in agriculture is the broomrapes (*Phelipanche* and *Orobanche* species*),* which is a limiting factor in the production of many crops, including processing tomatoes. Breeding for broomrape resistance is still one of the most effective management strategies for this root parasitic weed, but so far no broomrape resistance was found in any cultivated or wild tomato species. Recently we developed a tomato mutant, Sl-ORT1, which is characterized by its inability to produce and secrete strigolactones (SLs), which are secreted to the rhizosphere by almost all plants and serve as the natural stimulants for broomrape seed germination. Thus Sl-ORT1 was found to be highly resistant to various *Phelipanche* and *Orobanche* species. This mutant was obtained by fast-neutron mutagenesis that causes large chromosome deletions, which makes it difficult to identify the specific genes that are responsible for SLs deficiency. In contrast, ethyl methane sulfonate (EMS) mutagenesis causes point mutations that allow easier identification of the target genes. We therefore conducted EMS mutagenesis on 20,000 M82 tomato seeds. Screening the 2,200 second generation lines for broomrape resistance revealed 8 lines that were highly resistant and did not produce SLs. The total yield of the resistant lines was the same as in M82. This SLs deficiency also leads to a delay in fruit color change, but does not influence other fruit ripening characteristics, such as white shoulders, white veins, ripening uniformity and fruit hollowness. The soluble solid quantity (Brix) of mutagenized lines was either the same as in M82 or even higher. Interestingly, these mutant lines were more susceptible to soil diseases, which is consistent with the potential defensive role of SLs in the rhizosphere.

THE EFFECTS OF PHOSPHATE AND NITROGEN NUTRIENTS ON THE PRODUCTION OF STRIGOLACTONES IN ARABIDOPSIS

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Strigolactones (SLs) are host recognition signals for root parasitic plants and arbuscular mycorrhizal fungi in the rhizosphere. In addition, shoot branching is inhibited by SLs in host plants. Arabidopsis is widely used as a model plant in genetics and molecular biology, and in the elucidation of biosynthesis and signaling pathway of SLs. However it is difficult to analyze endogenous SLs and SLs exuded from roots of Arabidopsis, because Arabidopsis is a member of Brassicaceae which is a non-mycotrophic species and its SL production seems to be smaller than mycotrophic plants. So far we have shown that it was possible to analyze SLs using cultured cells of Arabidopsis that produce relatively a large amount of SLs. We have also demonstrated that root exudation of SLs increased under phosphate deficiency or both phosphate and nitrogen deficiency in other plants including Poaceae, Fabaceae, Solanaceae and Asteraceae. In this study, in order to understand the regulation of SL production in Arabidopsis, we investigated the effect of phosphate and nitrogen nutrients on SL production using Arabidopsis cultured cells. The suspension cultured cell T87 of Arabidopsis was provided from RIKEN BioResource Center of Japan. The T87 cells were cultured in different nutrient conditions and separated into cells and culture media before stationary phase. The neutral ethyl acetate-soluble fractions were extracted from the cells and media and used to examine a germination stimulant activity using seeds of root parasitic plant *Orobanche minor*. RNA was also extracted from the cells and the transcript levels of SL biosynthesis genes, *AtD27*, *MAX3* (*CCD7*), *MAX4* (*CCD8*) and *MAX1* (*CYP711A1*), were analyzed by RT-PCR. The germination stimulant activities and transcript levels were higher both in cells and medium when T87 was cultured in a medium containing 1.2 mM phosphate than 0.5 mM phosphate. Furthermore, those were higher in 60 mM nitrogen than 20 mM nitrogen. These data indicated that both phosphate and nitrogen deficiency do not promote but reduce SL production in Arabidopsis cultured cells. Currently, in order to know whether the same regulations work *in planta*, we are investigating the effects of phosphate or nitrogen deficiency on the branching of Arabidopsis plants.

DEBRANONES PARTIALLY AND SELECTIVELY MIMIC STRIGOLACTONE FUNCTION

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Strigolactones (SLs) are recognized as multifunctional chemicals, which behave as an allelopathic agent in the rhizosphere and as a plant hormone *in planta*. We have developed SL mimics with a focus on two of SL pleiotropic actions; one is seed germination stimulation of *Striga* spp. and the other is shoot branching inhibition as a plant hormone. In 11th WCPP in Italy, we presented our SL mimic "debranones" derived from phenol compound and butenolide, and showed 4BD (4-bromo debranone), one of debranones, strongly inhibits rice tiller buds outgrowth at 1 μM but doesn't stimulate seed germination of *Striga hermonthica* at the same concentration. This type of chemicals can be applied to control *Striga* infection by co-use with SL deficient mutants. 4BD recovers SL deficient phenotype of the mutants without stimulation of *Striga* seed germination. Next, we tried to find other type of debranones that mimic other SL actions. So, we synthesized more debranone derivatives and evaluated the activities of them by rice branching inhibition assay and *Striga* seed germination assay. Then, we found that substituent position on phenyl ring significantly affected the function-selectivity of debranones and that functional group mainly affected the activity of debranones. As a result of comprehensive structure-activity relationship study, the tendency of debranones to exert their activities was figured out as follows. 2,4-Disubstituted debranone compounds tend to exert strong branching inhibition activity but not strong germination stimulation activity. However, 2,5-disubstituted compounds showed comparable activity in both assays, and remarkably, 2,6-disubstituted compounds showed weak branching inhibition activity but notable stimulation activity for *Striga hermonthica* seed germination. In conclusion, we achieved to synthesize a series of debranones showing partial SL action separately. These results may suggest that there should be difference(s) in the mechanism of SL perception between seed germination stimulation of root parasitic plants and shoot branching inhibition of plants.

Acknowledgement: We would thank Prof. AG Babiker for a kind gift of *Striga hermonthica* seeds.

AN INVESTIGATION OF THE GENETIC BASIS FOR STRIGOLACTONE PERCEPTION IN PARASITIC PLANT GERMINATION

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Root parasitic plants (Orobanchaceae) have highly sensitive seed germination responses to strigolactones exuded into the rhizosphere by the roots of host plants. The basis of strigolactone recognition in parasite seed has remained elusive due to a lack of a genetically tractable system. *Arabidopsis thaliana* seed germination is enhanced by the synthetic strigolactone GR24, but it is more sensitive to karrikins, a family of structurally related lactone compounds found in smoke. Genetic studies performed in *A. thaliana* have demonstrated that two homologous a/b-hydrolase superfamily proteins, KAI2 and D14, are key components of strigolactone and karrikin signal transduction. KAI2 is necessary for promotion of seed germination by either strigolactones or karrikins, whereas D14 is required for control of axillary shoot branching by strigolactones. Recent biochemical and crystallographic evidence favor the idea that KAI2 and D14 act as receptors. **We hypothesize that KAI2 regulates seed responses to lactone-type germination stimulants in many angiosperms, but has evolved strigolactone specificity in the Orobanchaceae.** We identified an atypical expansion of *KAI2* gene copy number in *Triphysaria versicolor*, *Striga hermonthica*, and *Phelipanche aegyptiaca* (4 to 5 *KAI2* paralogs in each), whereas *D14* was retained as a single copy in these species. As gene duplication can enable protein subfunctionalization, we performed a molecular evolutionary analysis of *KAI2* orthologs across the angiosperms. This analysis indicated that *KAI2* paralogs in the Orobanchaceae have undergone faster rates of evolution and are likely under relaxed purifying selection. To functionally test the ligand-specificity of KAI2 in parasites, we introduced *KAI2* paralogs from *S. hermonthica* and *P. aegyptiaca* into the *A. thaliana kai2* mutant. Our preliminary analysis indicates that at least one *KAI2* paralog from each species restores the sensitivity of *kai2* seed to GR24, but not to karrikins. These data are consistent with a role for KAI2 in strigolactone recognition during seed germination of parasitic plants. Future work will determine whether individual KAI2 paralogs from parasites have unique specificities for subsets of strigolactones, which would enable a versatile host-recognition system.

Session 1: Genomics

Chairs

John Yoder and Jim Westwood

GENOME AND TRANSCRIPTOME ANALYSES OF STRIGA SPP.

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The obligate parasites *Striga* spp. are major constrains for crop production in sub-Saharan Africa and parts of Asia. Striga has a unique life cycle; germination requires plant hormones, so called strigolactones, germinated seedling grows toward host roots, and its radical tip deforms and develops a terminal haustorium. Understanding the molecular basis of its parasitism will give us a clue to develop efficient control methods. However, the molecular and genomic resources of Striga species have been still limited. Therefore we have performed the complete genome sequencing and large-scale transcriptome analyses of *Striga* spp.

Striga asiatica is an ideal material for complete genome sequence assembly because of its relatively small genome size (approx. 600 Mbp) and self-pollinating nature. We first cultured a single *S. asiatica* plant in axenic condition to obtain a large volume of homogeneous genomic DNA. Six paired-end and mate-pair libraries of different insert sizes were constructed and more than 200 Gbp sequences were obtained by the Illumina HiSeq2000 sequencer. These short reads were assembled and resulted in the scaffolds with N50 size of over 120 Kbp. About 50% of the genome is occupied by repeat sequences, suggesting *Striga* genome is expanded by repeat sequences like other plant species. The obtained genome sequence is being annotated using the information of closely related plant genomes and transcriptome sequences.

To understand molecular events offering during *Striga* infection, we have carried out a transcriptome analysis of *Striga hermonthica* during rice root infection. Total RNA was extracted at different infection stages, and RNA sequences were analyzed. Reference transcript sequences were constructed by *de novo* assembly, and short reads were mapped on the reference sequences to estimate expression levels. Gene ontology analysis suggests that genes encoding hydrolase enzymes are upregulated during host infection. The expression patterns were further confirmed by qRT-PCR and genes showing stage-specific expression during parasitism were identified.

Our genome and transcriptome data provide important resources towards complete understanding molecular and evolutional aspects of plant parasitism.

TISSUE SPECIFIC *DE NOVO* TRANSCRIPTOMICS IN THE PARASITIC OROBANCHACEAE

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The Parasitic Plant Genome Project is taking a comparative evolutionary approach to understanding parasitism in the Orobanchaceae. The project has generated more than 1.5 billion expressed sequence tags (ESTs) from three species, a facultative parasite (*Triphysaria versicolor*), a photosynthetically -competent obligate parasite (*Striga hermonthica*), and an obligate holoparasite (*Phelipanche aegyptiaca*). The *de novo* assemblies of these data represent all life stages from seed conditioning to flowering. We enhanced our sampling strategy via laser microdissection to provide a highly sensitive view of molecular processes at the parasite-host interface. In addition, ESTs (of whole-plant RNA) have been sequenced from the basal, non-parasitic Orobanchaceae species *Lindenbergia philippensis,* which is sister to all parasitic Orobanchaceae. The full genome of another nonparasitic relative, *Mimulus guttatus,* is a useful reference to characterize genome wide changes associated with the transition from autotrophy to heterotrophy. The sequences are provided as a resource to the public at http://ppgp.huck.psu.edu/. I will give several examples of how this massive sequencing effort is shedding light on the biology and evolution of Orobanchaceae, including identification of horizontal gene transfer events associated with parasitism and informatic strategies for identifying genes that may play important roles in parasite biology.

GENOME SCALE ANALYSIS OF LASER MICRO-DISSECTED TISSUES SHEDS LIGHT ON PARASITIC PLANT-HOST PLANT INTERACTIONS

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One of the largest and most economically important lineages of parasitic plants, Orobanchaceae, includes members ranging from facultative to obligate holoparasitic species. Parasitic members of Orobanchaceae growing in Africa and the Mediterranean include the devastating agricultural pests, witchweed (*Striga*) and broomrape (*Phelipanche*). The Parasitic Plant Genome Project (PPGP) is taking a comparative evolutionary approach to understanding parasitism in the Orobanchaceae. The project has generated more than 1.5 billion expressed sequence tags from three species, a facultative parasite (*Triphysaria versicolor*), a photosynthetically competent obligate parasite (*Striga hermonthica*), and an obligate holoparasite (*Phelipanche aegyptiaca*). The sequenced transcriptomes represent all life stages from seed conditioning to flowering. We enhanced our sampling strategy via laser microdissection to provide a highly sensitive view of molecular processes at the parasite-host interface. Using validated methods of *de novo* assembly to reconstruct the transcriptomes from Second Generation Sequencing data we explored laser microdissected tissues for each PPGP species. This revealed that stage-specific transcriptomes are enriched for genes families with unknown functions and that *T. versicolor* expresses genes in a host specific manner. This approach emphasizes that highly tissue-specific sampling, coupled with cost-effective RNA Sequencing, allows us to functionally isolate genes that are expressed in life stages and tissues critical to the parasite lifestyle.

AGROBACTERIUM RHIZOGENES TRANSFORMATION OF *ZEA MAYS*: A FUNCTIONAL GENOMICS TOOL FOR HOST-PARASITE INTERACTIONS

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Witchweed (*Striga* spp.) is a genus of parasitic weeds that debilitate cereal production in sub-Saharan Africa. All of the cultivated cereals are parasitized by one or more *Striga* spp. *Striga* infests two-thirds of the arable land of Africa and constitutes the biggest single biological cause of crop damage and loss of yield in Africa. There is no single effective method for controlling Striga. The best and most sustainable control measure would be to provide farmers with crop germplasm that is resistant to these parasites. However the biological processes underpinning host-parasite compatibility are poorly understood despite the fact that such information is vital for development of appropriate management strategies using both genetic modification (GM) and non-GM approaches. In order to exploit the rapidly emerging genome sequence and EST data available for Striga and other parasitic plant species and knowledge about changes in the expression of host and parasite genes during resistant and compatible interactions, a high throughput screen for gene function in cereals is required. Here we describe an efficient, fast transformation procedure for cereals. We used *Agrobacterium rhizogenes* strain K599 carrying a reporter gene construct, Green Fluorescent Protein (GFP), to generate transgenic composite maize plants that were challenged with the parasitic plant *S. hermonthica*. Eighty five percent of maize plants produced transgenic hairy roots expressing GFP. Consistent with most hairy roots produced in dicotyledenous species, transformed maize roots exhibited a hairy root phenotype, the hallmark of *A. rhizogenes* mediated transformation. Transgenic hairy roots were readily infected by *S. hermonthica*. There were no significant differences in the number and size of *S. hermonthica* individuals recovered from either transgenic or wild type roots. This rapid, high throughput, transformation technique will advance our understanding of gene function in parasitic plant-host interactions.

TRANS-SPECIFIC GENE SILENCING: A BIOLOGICAL STRATEGY TO CONTROL PARASITIC WEEDS?

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We are assessing a strategy for engineering crop resistance against parasitic weeds that is based on interfering RNA (RNAi) constructions transcribed in host roots and designed to be toxic to invading parasites. We made five hairpin RNAi constructions designed to inhibit the cytosolic acetyl-CoA carboxylase (ACCase) gene of *Triphysaria versicolor* and introduced these into *Medicago truncatula* roots by *Agrobacterium rhizogenes* transformation. Transgenic *Medicago* roots were recovered after transformation with four of the five ACCase constructions and infected with *Triphysaria*. Within a week of infection, the viability of *Triphysaria* roots was reduced up to 80% when parasitizing a host transgenic for any of the ACCase hairpins. Parasitizing roots continued to grow when the plates were supplemented with exogenous malonate, suggesting that the root lethality is associated with a deficiency in the ACCase enzyme. RT-PCR showed that ACCase transcript levels were reduced in *Triphysaria* roots attached to the transgenic hosts. Northern blot analysis identified an ACCase specific ~ 21 nt RNA molecule in transgenic *Medicago* roots and in roots of *Triphysaria* attached to them. This work shows that hairpin ACCase constructions expressed in *Medicago* roots can inhibit root growth of parasitizing *Triphysaria*. Probably the most critical parameter to the deployment of this strategy is the specificity of the oligonucleotide toxin for the parasite transcripts. We found that one of the hairpin ACCase constructions could not be transformed into *Medicago* unless grown in media supplemented with malonate. Quantitative RT-PCR showed that *Medicago* ACCase transcript levels were reduced in malonate recovered transgenic roots. Apparently this construction contains sequences of sufficient similarity to *Medicago* transcripts that they are toxic to *Medicago* roots. The extensive similarities between gene sequences in different Orobanchaceae species make us hopeful that these constructions will be effective against *Striga* and *Orobanche*. However there may be differences in ACCase metabolism, gene copy number, or RNAi transport mechanisms that will require additional tuning of this strategy as an effective field measure.

GENETIC ASPECTS OF STIMULANT SPECIFICITY IN *OROBANCHE* SEED GERMINATION

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The seeds of obligate root-parasitic Orobanchaceae are totally dependent on chemical stimulation from host roots for the onset of germination, which increases the chance for the non-photosynthetic holoparasites to find a potentially nourishing host in their vicinity immediately after germination. Host specificity is often determined by seed response to specific germination stimulants. The aim of this study is to explore the inheritance of this unique specificity to germination stimulants. For this we analyzed the segregation of stimulant specificity in seeds of hybrid progenies derived from crosses between *O. cernua* that responds to strigolactones from tomato root exudates, and *O. cumana* that responds to dehydrocostus lactone, a sesquiterpene lactone from sunflower root exudates. Segregation of seed response to the two groups of germination stimulants was found in F_2, indicating that the stimulant specificity is genetically controlled in the embryo. Our previous findings showed that spontaneous germination (germination without chemical stimulation) develops in F_3 seed families and not in F_2, which indicates the involvement of the seed perisperm (a maternal seed tissue) in germination control. Based on the combined understanding of seed anatomy and germination genetics, a model for the mechanism of germination control in these seeds will be suggested.

DE NOVO TRANSCRIPTOME ASSEMBLY OF *CUSCUTA PENTAGONA* AND BIDIRECTIONAL MOVEMENT OF mRNA BETWEEN HOSTS AND PARASITE USING HIGH-THROUGHPUT SEQUENCING

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Cuscuta pentagona (lespedeza dodder) haustoria link to hosts via symplastic connections that allow movement of macromolecules between the two plants. We are particularly interested in host-parasite trafficking of RNA and have addressed this subject using high-throughput sequencing as a cost-effective tool to obtain high detection sensitivity and dynamic range of gene expression. In the process of developing a comprehensive profile of mobile RNAs and gaining insight into the mechanisms regulating cross-species transfer, we generated a *de novo* transcriptome assembly of *Cuscuta* and mobile/non-mobile transcriptomes for the host-*Cuscuta* system. *Cuscuta* was grown on *Arabidopsis* or tomato hosts because their sequenced genomes facilitate bioinformatic identification of transcripts moving between the plants. We focused on three regions of interaction: *Cuscuta* stem near host attachment site, the region of attachment between *Cuscuta* and the host plant, and the host stem adjacent to the attachment site. High stringency mapping and pre-processing of 1.5 billion Illumina reads (75 and 100 bp long) from host and *Cuscuta* tissues allowed us to determine the identity of the reads. For the mobile transcriptome analysis, we detected a large number that correspond to host sequences in parasitic tissue with 11,000 *Arabidopsis* transcripts and 3,000 tomato transcripts found in the parasite. *De novo* assembly of *Cuscuta* sequences using the Trinity assembler generated 252,580 contigs with mean size of 1,164 bp, which were compared with sequences in GenBank for annotation and evaluation of sequence quality. These represent the *Cuscuta* stem and haustorium transcriptomes. The *de novo* assembly of *Cuscuta* data provided a good reference dataset to confirm bidirectional movement of mRNAs between hosts and parasite. Also, approximately 3,000 *Cuscuta* unigene transcripts were confirmed to be mobile into both hosts. Mobile RNAs from hosts and parasite were functionally categorized using GO-slim terms, and all represent a broad cross-section of the transcriptomes. Beyond understanding the mobile transcriptome, these data provide a starting point for understanding parasite gene expression, including alternative splice sites, novel genes and potential non-coding RNAs. This will facilitate studies of the *Cuscuta* haustorium and comparisons to transcriptomes from other parasitic plant species.

CHARACTERIZATION OF MOBILE RNA FROM HOSTS INTO *CUSCUTA PENTAGONA*

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The cross-species movement of mRNA between *Cuscuta pentagona* and its hosts has been documented, but not characterized quantitatively or with attention to uptake patterns and fate of specific mRNAs. We used a combination of quantitative Real-Time PCR and RNA-Seq approaches to identify and characterize mobile transcripts from tomato and *Arabidopsis* hosts into *C. pentagona*. The result is a variety of behaviors by different transcripts that defy explanation by a single pathway or mechanism. For example, tomato transcripts of Gibberellic Acid Insensitive (*SlGAI*) and Cathepsin D Proteinase Inhibitor (*SlPI*) differed significantly in rate of uptake into the parasite, but were then distributed over the length of the parasite shoot. Similarly, the *Arabidopsis* mobile RNAs of Translationally Controlled Tumor Protein (*AtTCTP*), Auxin Response Factor (*AtARF*) and a Salt-inducible Zinc Finger Protein (*AtSZF1*) also differed in their mechanisms of uptake between host and parasite. Although it is possible that the parasite forms connections to both the phloem and parenchyma cells of the host, the known phloem-mobile RNAs (*SlGAI* and *AtTCTP*) showed uptake patterns that differ from each other as well as from other RNAs that have not been reported to be phloem mobile (*SlPI* and *AtSZF1*). RNA-seq analysis suggests that the transfer of mRNA from host to parasite follows at least two major pathways, with substantial variation among transcripts. Furthermore, host transcripts are degraded or processed in the parasite, as the *SlPI* transcript concentrations in the parasite were mostly lost from the parasite within eight hours of detachment from the host. For *AtSZF1*, it appears that the 3' end of the transcript persisted in the parasite longer than the 5' end. Taken together, the uptake and distribution of host mRNAs into *C. pentagona* varies among mRNAs and suggests that mRNAs traffic into the parasite via multiple routes, and may be regulated by other mechanisms that lead to selective uptake and mobility between host and parasite.

AGROBACTERIUM-MEDIATED TRANSFORMATION OF *PHELIPANCHE RAMOSA*

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Parasitic *Phelipanche* and *Orobanche* (broomrapes) spp. are economically important parasitic plants, They can heavily damage many agricultural crops. *Phelipanche ramosa* (L.) Pomel (syn. Orobanche ramosa (L.)) and P. *aegyptiaca* parasitize mainly tomato, rapeseeds, carrot, potato, tobacco, eggplant and many others. *O. cumana* Wallr. parasitizes sunflower in eastern Europe around the Black Sea, in Spain and in Israel. *O. crenata* is a widespread parasite of legumes all around the Mediterranean. Their main distribution is throughout the Mediterranean region, in Eastern Europe and India. Conventional methods to control parasitic Phelipanche and Orobanche spp. are not effective. It is very difficult to control these root parasites, because their roots are physiologically connected to the roots of host plants to get all nutrients and water needed. Established physiological connection between the parasite and the host reduce possibility of broad use of herbicides for weed control. The most of damage is done before shoots emerge above the soil and before the infection is detected. At present, combination of several methods is used to reduce parasitism of Phelipanche and other parasitic plants. The new perspective approach focuses on identification and description of molecular and biochemical processes involved in the establishment of connection between the parasite and the host at very early stages of their interaction.

In the present study, we show (1) establishment of in vitro cultures of *Phelipanche ramosa* and (2) preliminary results on *Agrobacterium*-mediated transformation of *P. ramosa* using red fluorescent protein (dsRED) as a marker. Transgenic *P. ramosa* tissue was able to develop haustoria-like structures on roots of host plants in *in vitro* conditions.

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TRANSCRIPTOMICS IN PARASITE DEVELOPMENT OF *STRIGA HERMONTHICA*

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Despite the agricultural impact of parasitic plants, the molecular and developmental processes underlying the parasite development are not well understood. Parasitic plants develop specialized organs called haustoria to attach to host tissues, establish vascular connection, and extract host nutrients and water for growth and reproduction. A key to develop novel strategies for controlling parasitic plants is to understand the molecular mechanism of host-parasite associations. It is therefore of great interest to advance our understanding of plant parasitism and to apply this knowledge towards the development of resistant crops. Here we performed transcriptome sequencing (RNA-seq) analysis on sequential parasitic stages of *Striga hermonthica* in rice. The transcripts were assembled de novo, analyzed by BLAST to the plant databases to determine the transcriptomic profile of *S. hermonthica*. Our multidimensional scaling and principal component analyses successfully dissected the dynamics of gene expression along the parasite development. Self-organizing map clustering combined with gene ontology analysis revealed expression patterns of several key genes and their biological functions. Especially gene co-expression network analysis identified key candidate genes, which could be potential targets for the parasite-resistant crops.

Session 2: Biology and Biochemistry

Chairs

Philippe Delavault and Philippe Simier

CYP707A1, AN ABA CATABOLIC GENE, IS A UBIQUITOUS COMPONENT OF PARASITIC PLANTS SEED GERMINATION IN RESPONSE TO VARIOUS GERMINATION STIMULANTS

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Germination of root parasitic plants seed occurs, after a moist conditioning period, by chemical germination stimulants (essentially strigolactones) secreted into the rhizosphere by host plant roots. Using *Phelipanche ramosa* as plant model, we demonstrated that seeds require a conditioning period of at least four days to become receptive to the synthetic germination stimulant GR24. Using a cDNA-AFLP strategy, 58 transcripts-derived fragments (TDFs), whose expression pattern changed upon GR24 treatment, were isolated. Among the isolated TDFs, two up-regulated sequences corresponded to *PrCYP707A1*, encoding an ABA 8'-hydroxylase, an enzyme involved in abscissic acid (ABA) catabolism. While this gene was expressed at low levels during the conditioning period, an initial decline in ABA levels was recorded, that could essentially be attributed to diffusion out of the seed during imbibition. After conditioning, GR24 application triggered a strong up-regulation of *PrCYP707A1* during the first 18h, followed by an 8-fold decreased in ABA levels detectable 3 days after treatment. Concomitant treatments of conditioned seeds with GR24 and exogenous ABA, or Abz-E2B, a specific inhibitor of CYP707A, caused inhibition of germination. These results demonstrated that germination occurs after a dormancy release of the seeds by ABA catabolism mediated by the GR24-dependent activation of *PrCYP707A* gene. In addition, *in situ* hybridization corroborates the putative location of cells receptive to the germination stimulants in seeds. We then evaluated whether *CYP707A* overexpression constitutes a ubiquitous mechanism in parasitic plant seed germination induced by GS. First, responses of *P. ramosa*, *O. cumana*, *O. minor*, and *S. hermonthica* seeds to different GS - the synthetic strigolactone GR24, the sunflower sesquiterpene lactone dehydrocostus lactone (DCL) and the 2-phenylethyl isothiocyanate (ITC) present in the rhizosphere of oilseed rape - were analyzed. The seeds displayed differential response patterns according to the species, the stimulants and the applied concentration. Thus, the four species germinated in response to GR24, only the three broomrape species responded to DCL, and only *P. ramosa* germinated in response to ITC. Whatever the GS and the species, when germination was triggered, a *CYP707A* overexpression was observed. These results revealed the ubiquitous key role of *CYP707A* in parasitic plant seed germination triggered by GS.

INHIBITORY EFFECT OF NOJIRIMYCIN ON GERMINATION AND SUGAR METABOLISM OF A BROOMRAPE

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Root parasitic weeds, *Striga* spp. and *Orobanche* spp. cause serious damage to worldwide agriculture. These parasites affect 300 million people and cause damage of 7 billion $US per year. Therefore, an effective strategy for parasite control is desired. Since the life cycle of the parasites is significantly different from that of host plants, understanding of the parasite-specific biological event is important for design of selective control strategies. The root parasitic weeds require host-derived strigolactones for seed germination. We focused on this unique germination process to find biological event specific to these species. A metabolome analysis using germinating *O. minor* seed revealed that a trisaccharide immediately decreased and monosaccharides increased following GR24 induced germination. To elucidate the importance of the metabolism related to this trisaccharide, we studied the effect of some glycosidase inhibitors on the germination. Consequently, nojirimycin bisulfite (NJ) selectively inhibited the germination of the parasites. Additionally, the trisaccharide was consumed after GR24 treatment in both NJ-treated and germinating seeds, on the other hand, monosaccharides levels decreased and the amounts of a disaccharide significantly increased in the NJ-treated seeds compared with those in the germinating seeds. From these results, we hypothesized that NJ inhibits the germination by suppressing the supply of monosaccharides. Therefore, We studied whether addition of exogenous sugars to NJ-treated seeds recovers the germination rate. As a result, a significant recovery of the germination rate was observed when a particular monosaccharide was applied, strongly suggesting that NJ inhibits the germination by blocking the supply of monosaccharide. A part of this study was supported by NEDO (to AO).

ASPECTS OF GLYPHOSATE MECHANISM IN EGYPTIAN BROOMRAPE CONTROL

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Egyptian broomrape (*Phelipanche aegyptiaca*) is an obligate root parasite that poses a severe threat in the Mediterranean agriculture. *P. aegyptiaca* tubercles function as a strong sink and draw water, assimilates and xenobiotics from the host plant. Glyphosate is a non-selective herbicide that inhibits 5-enolpyruvyl shikimate -3-phosphate synthase (EPSPS), a key enzyme in the shikimate pathway and in aromatic amino acids (AAA) biosynthesis. *P. aegyptiaca* is efficiently controlled by glyphosate when applied on the foliar of its host plant. The general notion suggests that plants in general are controlled by AAA deficiency, however, the active presence of EPSPS was not yet shown in *Phelipanche/Orobanche* spp. and since the parasite receives all of its nutritional requirements from the host, the mechanism for its control is not clear. The objective of the current study was to examine EPSPS activity in *P. aegyptiaca*. In order to isolate glyphosate effect on the parasite from its effect on the host, we have used glyphosate resistant tomato (GRT) as a host plant, thus delivering the herbicide to the parasite tissue without affecting its nutrients supply from the host. EPSPS inhibition was evaluated by shikimate accumulation in the plant tissues. Shikimate accumulation was detected in *P. aegyptiaca* tubercles following glyphosate application, grown either on GRT host or on a sensitive tomato. GRT shoot apex did not accumulate shikimate suggesting that the shikimate found in the parasite is endogenous. High levels of shikimate found in the parasite indicate the presence of an active EPSPS in its tissue. This finding also implies that *P. aegyptiaca* might have its own mechanism for synthesizing AAA. The inhibition of EPSPS in plant tissue is thought to cause a carbon flux into the shikimate pathway. Such an event might affect the entire carbon metabolism of the plant. In a previous study we have found that glyphosate inhibited phloem translocation to *P. aegyptiaca*. We hypothesize that glyphosate disrupts carbon metabolism in the parasite by inhibiting EPSPS, weakening the parasite sink strength and therefore interrupts with solutes transport that eventually leads to its death. Experiments to examine this hypothesis are now in progress.

ANATOMICAL AND FUNCTIONAL CHANGES ON THE HOST WOOD CAUSED BY THE INFESTATION OF *PHORADENDRON CRASSIFOLIUM* (VISCACEAE)

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This study aimed to assess whether the presence of the parasite *Phoradendron crassifolium* caused anatomical and/or functional changes in the wood structure of *Tapiria guianensis*. We collected 20 branches with approximately the same diameter. Half of the branches were non-parasitized and the other half were parasitized. Right after their removal, all branches were quickly submerged in filtered water, and then they were sectioned and had their vessel openings de-obstructed with sharp blades. All branches were then carefully transferred to safranin solution (0.01%) for about 5 hours. After removal from the solution and air-drying, the infested branches were divided into portions bellow and above the site of parasite attachment, thus creating two groups ("upstream" and "downstream"). The non-parasitized branches were also divided at similar positions, also creating two groups ("proximal" and "distal"). The use of such groups allowed us to compare segments with the same distance from main trunk and wood maturity. Standard wood-anatomical methods for both macroscopical and microscopical analysis were used to evaluate several anatomical features. Statistical analysis (nested ANOVA) indicated that parasitized branches had about 54% more non-functional (embolized) vessels/mm². This alteration could cause a reduction in total sap conduction, which may explain the occurrence of death at the downstream position of the parasitized branches. With respect to the wood anatomy of the host we observed that parasitized branches have vessels with smaller diameter and higher density. These branches also showed an increase in the density of grouped-vessels and an increase in ray width and height. All such effects were more extreme at the downstream portion of the parasitized branch (above parasite's point of attachment). Altogether these anatomical changes seem to be an attempt of the host to compensate the loss of hydraulic conductivity related to the presence of the parasite. Therefore we conclude that the parasitism by *Phoradendron crassifolium* can act as a biotic factor altering the wood of *Tapirira guianensis* in two ways*:* 1) causing more embolisms in the conducting system and, as a consequence, a possible reduction on xylem sap influx (short-term effect); 2) as a response to the loss of conductivity, where cambium reacts producing wood with more cavitation-resistant features, such as thinner vessels in higher density and more grouped (medium-term effect).

PLANT DEFENCE RESPONSES AGAINST ROOT PARASITIC PLANTS

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Root parasitic plants of the family Orobancheaceae - including *Striga*, *Orobanche* and *Phelipanche* spp - cause severe damage to important agricultural crops worldwide. These parasitic weeds are difficult to control since they are intimately associated with the host root, and most of their lifecycle occurs belowground. This fact makes the diagnosis of infection difficult, and normally when irreversible damage has been caused to the crop. Therefore, new and more effective control strategies against these parasitic weeds should be focus on the initial stages of the interaction. Using tomato-*Phelipanche ramosa* as a model system, we have explored the host response during the early stages of parasitic infection by monitoring the expression defence marker genes. For that purpose, three different colonization stages were selected and local and systemic roots were analyzed by quantitative real time PCR. The jasmonate (JA)-related genes *LoxD* and *Pin II* were induced locally and systemically along the time. Conversely, the expression of the salicylate (SA) marker gene *PR1a* was reduced in both tissues. These preliminary results suggest that the host plant active the JA-dependent responses in order to avoid the colonization, and that the attenuation of the SA-dependent signalling pathway is required for a successful infection of the parasite.

Strigolactones (SLs) are multifunctional molecules classified as a new class of plant hormones regulating different processes in the plant. SLs are also crucial cues in host-root parasitic plant interactions in the rhizosphere. They induce germination of the parasitic weed seeds, thus enabling plant infection. The expression of the SLs biosynthesis genes CCD7 and CCD8 was also analyzed in our tomato-*P. ramosa* system. The results point to an additional involvement of SLs during the initial stages of colonization. These possible new roles for SLs will be discussed.

THE CHEMICAL NATURE OF PARASITIC AND MYCOHETEROTROPHIC METABOLISM INVOLVES THE RECONFIGURATION OF SUBSTRATE USAGE IN ORDER TO SUSTAIN THE TRICARBOXYLIC ACID CYCLE

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Although the majority of terrestrial plants make use of beneficial symbiotic associations, certain plants have adapted alternative strategies to obtain nutrients and carbohydrates from the environment. These adaptations involve both utilization and metabolism from sedentary/decaying (saprophytic) or living (parasitic) sources. The primary carbon metabolism of these lifestyles is poorly characterized on a molecular or biochemical level. Here I investigated the interesting adaptive mechanisms of the parasitic lifestyle of both (true) parasitic (plant host) or mycoheterotrophic (fungal host) plants concerning their unique carbon regulatory strategies; and reveal that, through the power of metabolite profiling, a unique mode of regulation for the tricarboxylic acid (TCA) cycle that attain the *status quo*. While low levels of organic acids were detected in the parasitic plants; these exhibited increased levels of branched chain amino acids, which serve as an alternative respiratory substrate. Furthermore, high levels of polyol sugars (*myo*-inositol and trehalose) were detected with levels of sucrose, fructose and starch significantly reduced. Taken together, this suggests that the primary carbon metabolism of parasitic plants is uniquely adapted to sustain the resulting association in favour of exclusive exploitation.

PARASITIC PLANTS IMBIBE HOST PLANT TOXINS THAT INFLUENCE INSECT HERBIVORES

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Many insect herbivores sequester plant-derived chemical toxins within their own bodies as a defense against predators. Parasitic plants represent another class of plant-feeding organisms and face similar challenges from natural enemies at higher trophic levels. Yet, little research has explored the potential role of host-plant-derived defenses in interactions between parasitic plants and insect herbivores. A few studies have shown that the resistance of parasitic plants to herbivory can be influenced by the identity of the host plant. For example, aphids feeding on vines of the parasitic weed dodder (genus *Cuscuta*) vary in their success as a function of dodder's host plant species. Whereas most aphid species perform relatively poorly on dodder that is grown on turnip (*Brassica rapa*)—a plant defended against insects by glucosinolate toxins—one aphid species that is well adapted to tolerate these toxins (*Brevicoryne brassicae*) performs optimally on turnip-hosted dodder. In light of these observations, we hypothesized that aphids are influenced by the translocation of glucosinolate defenses from the host plant to the parasite. Here we discuss recent biochemical analyses (high pressure liquid chromatography) demonstrating that glucosinolates indeed move readily into dodder vines from host plants in the cabbage family (Brassicaceae). To assess the effects of these toxins on dodder's herbivores in vivo, we grew the parasite on three lines of *Arabidopsis thaliana* that vary in glucosinolate production and then presented these dodder vines to two classes of dodder-feeding insects (aphids and *Lygus* bugs) in choice and performance assays. Our findings confirm that host-derived glucosinolates enhance or inhibit aphid reproduction in an aphid-species-specific manner; additionally, we found that these toxins stimulate oviposition by *Lygus* bugs. Thus, the transfer of host toxins into parasitic plants can significantly influence interactions among plant parasites and their insect communities. This work has significant implications for the ecology of parasitic plants and their insect herbivores, as well as for the management of some of the world's most devastating agricultural weeds.

NUTRIENT REQUIREMENTS DIFFER IN TWO *PEDICULARIS* SPECIES IN THE ABSENCE OF A HOST PLANT: IMPLICATION FOR DRIVING FORCES IN THE EVOLUTION OF HOST PREFERENCE OF ROOT HEMIPARASITIC PLANTS

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Facultative root hemiparasitic plants generally have wide host range, but in most cases showing obvious host preference. The reasons for the marked difference in growth performance of the hemiparasites when attached to different hosts are not fully understood. In this study, we tested the hypothesis that hemiparasites showing preference for different hosts have different nutrient requirements. Two facultative root hemiparasitic *Pedicularis* species (*P. rex* and *P. tricolor*) with different host dependency and preference were used to test their responses to inorganic solutes. Effects of nitrogen, phosphorus, and potassium on growth of the hemiparasitic plants not attached to a host were determined, using an orthogonal design in pot cultivation under greenhouse conditions. Variables including biomass, shoot nutrient concentration, root: shoot (R:S) ratios, and the number of haustoria were measured. As in autotrophic plants, nutrient deficiency reduced dry weight (DW) and nutrient concentrations in the root hemiparasites. N and P significantly influenced growth of both *Pedicularis* species, while K availability influenced only shoot DW of *P. rex*. Nitrogen had far more effect on growth of *P. rex* than on *P. tricolor*, while P deficiency caused more marked growth depression in *P. tricolor* than in *P. rex*. *Pedicularis rex* grew faster than *P. tricolor* in a range of nutrient supplies. Different patterns of biomass allocation between the two *Pedicularis* species were observed. While *P. rex* invested more into roots (particularly fine rootlets) than into shoots, the number of haustoria produced by this species was relatively much lower than by *P. tricolor*, which had a much lower R:S ratio. The two *Pedicularis* species differ in nutrient requirements and biomass allocation. Distinct interspecific traits in growth and nutrient requirements can be driving forces for the differential interactions between the hemiparasites and their hosts.

FUNCTIONAL CHACTERIZATION OF A β-MANNOSIDASE INVOLVED IN THE EARLY GERMINATION PROCESS OF *OROBANCHE MINOR*

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Orobanche minor is a root-parasitic plant of the Orobanchaceae. The root-parasitic weeds in the family attack many crops and are responsible for severe losses in the worldwide agriculture. Despite extensive studies no successful strategy for controlling its proliferation has been reported until now. In this context we focused our effort on the understanding of the unique germination process of this holoparasite, which could enable us to develop selective control strategies. The metabolic profiling of *O. minor* germinating seeds combined to a careful analysis of EST data libraries has lead us to study in detail a β-mannosidase homolog (*Om*βMan1) which is exclusively expressed in the early stages of the germination process. Therefore, molecular cloning of the full length cDNA of this enzyme has been performed and the corresponding recombinant enzyme has been expressed in *E. coli* for its functional analysis. The subcellular localization study of this enzyme by GFP-imaging in BY-2 cells revealed that this β-mannosidase is a secreted enzyme, targeted to the cell wall. Fusion/deletion experiments have shown that this localization is driven by an N-terminal targeting peptide, which is both necessary and sufficient. This targeting is also in good agreement with the presence of the substrate of this enzyme in this compartment. Such a key enzyme specifically involved in the early germination process of *O. minor* could be a novel target for selective and effective control of this parasite. Metabolic engineering approaches like RNA interference in the host plant *Trifolium pratense* (or related species) will also constitute a valuable tool to better understand the role of this enzyme in the host-parasite interaction and could even be a way to block the penetration of haustorium into the host root. Parts of this work were financially supported by JSPS (GG and TW), NEDO (AO) and Asahi Glass Foundation (AO).

CHARACTERIZATION OF THE GENES ENCODING SUCROSE TRANSPORTERS AND SUCROSE-DEGRADING ENZYMES IN THE PARASITIC PLANT *PHELIPANCHE RAMOSA*

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Phelipanche ramosa (branched broomrape) is a harmful root holoparasitic weed in major crops (especially tomato, tobacco, hemp, oilseed rape) in the Mediterranean area. It grows by connecting to the phloem vasculature of the host plant via a haustorium, thus allowing access to water and nutrients, notably sucrose. Considering the important role of this diholoside in the functioning of the plant-parasitic plant interaction, we investigated the sucrose unloading pathways and genes potentially involved in sucrose transport and utilization in *P. ramosa*. First, we used the phloem tracer carboxyfluorescein (CF) to demonstrate the existence of a symplasmic continuum at the host-parasite interface and, that the phloem unloading in various sink tissues of *P. ramosa* is mainly an apoplasmic type. Then, we identified the main actors involved in sucrose transport (SUT = sucrose transporter) and sucrose metabolism (invertases and sucrose synthases). Using molecular approaches, immunolocalization and *in situ* hybridization, we demonstrated the implication of some of these actors in major processes, such as the long-distance transport of sucrose and its unloading in sink organs (PrSUT1 and PrSUT3), hexose accumulation *via* a vacuolar acid invertase (PrSAI1), the tracheid differentiation and the starch synthesis *via* sucrose synthases (PrSUS1 and PrSUS2, respectively). We used GFP as a gene fusion in *A. thaliana* protoplasts to confirm the respective plasmalemmic and tonoplastic locations of PrSUT1 and PrSUT3. We hypothesize that PrSUT1, PrSUT3 and PrSAI1 act in concert in sink cells for sucrose unloading, sucrose transfer into vacuoles and then sucrose hydrolysis into hexoses. All these genes/proteins that are active in the sink strength of the parasitic plant are good targets in biotechnological strategies to control broomrapes. Trans RNA silencing strategies from *Arabidopsis* plant to *P. ramosa* are in progress in the lab for the functional analysis of the candidates genes.

GENETIC AND PHENOTYPIC DIVERSITIES IN THE PARASITIC SPECIES *PHELIPANCHE RAMOSA*

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Majority of *Orobanche* and *Phelipanche* species (broomrapes) relies on a restricted range of hosts and is usually present in natural ecosystems. However, by acting as annual weeds, usually with a much wider range of hosts, some species are now frequently observed in man-made ecosystems (agrosystems). Their prevalence can therefore have a devastating impact on crops. Amongst them, *Phelipanche ramosa* is by far the most widespread. This parasitic weed has found ideal growing conditions around the Mediterranean and in many Western European countries. This prevalence is linked to the increase in the cultivation of susceptible plants such as those in the *Solanaceae* family (tomato, tobacco etc.). Accidental introduction of broomrape has been discovered in Eastern Europe, Southwest Asia, South Africa, Chile and California. *P. ramosa* has an extremely wide range of possible host plants: many different farmed species and over seventy wild species. High infestations in rapeseed (*Brassicaceae*) fields are reported in France since almost two decades, and more recently in many other European countries. In France, winter oilseed rape has even become the primary host of *P. ramosa*, along with hemp and tobacco. Three French pathotypes can be discriminated using molecular plastidial and mitochondrial markers, geographic distribution and phenotypic indexes including susceptibility of seeds to germination stimulants and aggressiveness towards a set of host species. Thus the type I differs strongly from the two others by a strong prevalence in the Western Central of the country, then parasitizing a large range of weedy and farmed species (mainly winter oilseed rape), an enhanced susceptibility to strigolactones and no parasitism of hemp. How can we explain this scenario? This question is at the origin of a more extensive project with the objective to set the populations of *P. ramosa* on rapeseed (type I) in a collection which characterizes the whole distribution area and the range of hosts within the species as explicitly as possible. This project stems from a number of collaborators belonging to the “International Parasitic Plants Community” (opened circle of collaborations) for providing kindly their local *P. ramosa* populations (or very closed species), and private funds (SOFIPROTEOL, France).

COMPARATIVE PHENOLOGY OF TWO PARASITIC PLANTS OF THE GENUS *STRUTHANTHUS* (LORANTHACEAE) INFESTING TWO DIFFERENT HOSTS

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Loranthaceae is the best known and widespread group among mistletoe families in Brazil, comprising 73 genera. Among these, the genus *Struthanthus* is the most diverse and well-studied. Studies focusing on *Struthanthus* species deal mostly with its embryology, taxonomy and anatomy, leaving a gap in the relationship between host and parasite. The present study aimed to analyze the phenology of *S. vulgaris* and *S. flexicaulis*. The latter was observed when infesting a deciduous host (*Tipuana tipu*) and a non-deciduous one (*Psidium guajava*) while *S. flexicaulis* was only observed when infesting a non-deciduous host (*Ligustrum lucidum*). Correlations between parasite and host phenology and correlations with environmental parameters were also assessed. The events observed in each species were flower/fruit emergence, leaf changing and epicortical-root growth. Each of these events was divided into phases and the intensity of each phase was evaluated using a semi-quantitative method. The results showed that parasite leaf changing phenology is correlated to the deciduous nature of the host. During the observation period *T. tipu* showed maximum leaf loss and fruit maturation from May to August. While infesting this deciduous host *S. vulgaris* shows an increase of about 10% in the intensity of senescent leaves (from September to November). These results suggest that hormones related to leaf senescence and fruit maturation (e.g., ABA) may flow from the host to the parasite and influence its phenology during the following months. With respect to the comparison between *S. vulgaris* and *S. flexicaulis* the results showed that the two parasites exhibit opposite phenologies for their reproductive phases. The anthesis of *S. vulgaris* took place between April and June. In *S. flexicaulis* the anthesis took place from November to January. The fruit dispersion in *S. vulgaris* occurred from September to November. In *S. flexicaulis* fruit dispersion occurred from May to July. This can indicate a case of niche-partitioning when the parasitic species avoid competition for the disperser bird species. With such phenological patterns the bird dispersers (e.g., sabiá – *Turdus rulfiventris*, Turdidae), have a wider period of fruit availability, consequently enhancing its fidelity to this resource. Also, corroborating these results, the correlations with environmental parameters (α=0.05) showed a negative correlation between the solar irradiation and the end of flower presenting in *S. vulgaris* and a positive correlation for the same parameters in *S. flexicaulis*. This can indicate that the opposite season distribution of flowering on these two plants may be related to the annual variation on solar irradiation.

METABOLOMIC ANALYSIS OF EARLY STAGES OF *PHELIPANCHE AEGYPTIACA* DEVELOPMENT

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The developmental transition from free-living parasite seeding to attached parasite encompasses the major events in parasitism. Specifically, this transition includes the initiation and development of the haustorium, penetration of host tissues, and linkage to host vasculature such that the parasite can act as a sink for host resources. The transcriptional events associated with specific stages in this process have been characterized in the Parasitic Plant Genome Project for three parasitic Orobanchaceae, *Triphysaria versicolor*, *Striga hermonthica*, and *Phelipanche aegyptiaca*. However, understanding of this developmental transition will be facilitated by characterization of metabolomic profiles of these same stages, with the ultimate goal of correlating the parasite metabolomes with transcriptomes. In pilot studies using *P. aegyptiaca*, metabolic profiles have been generated from key stages, including parasite seedlings before host attachment, seedlings in the process of making host attachments, and developing parasite tubercles. For each stage, tissues (1 mg total dry wt per replicate) were extracted and metabolites separated by gas chromatography and analyzed on a single quadrupole mass spectrometer. Free-living and parasitic stages of *P. aegyptiaca* showed large differences in relative levels of a number of major metabolites. Among the major sugars fructose, glucose, and sucrose, and the carboxylic acid citrate were substantially higher in the expanded tubercle than in the seedling. In contrast, relative levels of mannitol were highest in free-living stages. This work is a starting point for more detailed metabolic analyses and comparisons among related parasites that will lead to the identification of key parasite metabolic pathways and new targets for disruption by control strategies.

Session 3: Ecology and Population Biology

Chairs

Yaakov Goldwasser and Jonne Rodenberg

THE DISTRIBUTION AND EVOLUTION OF THE GENUS *CYNOMORIUM*

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Cynomorium is the only genus in the Cynomoriaceae family and includes two species: *Cynomorium coccineum* and *Cynomorium songaricum*. *Cynomorium spp.* were used as medicine and food for thousands of years. In the 16th Century, the Knights Hospitaller of St. John operated a hospital in Jerusalem and learned the medicinal qualities of *C. coccineum* from local Muslim physicians. The Arabs called *C. coccineum* "treasure of drugs" and it was mainly used to treat apoplexy, venereal disease, high blood pressure, vomiting, irregular menstrual periods and as a contraceptive. *C. songaricum* was described by the famous Chinese pharmacists Shizhen Li in his "Compendium of Materia Medica" (1578); it was mainly used to cure fullness of the abdomen after eating, dyspepsia, "kidney deficiency", lumbago and diarrhea. There was an interesting legend relating to an old Chinese town – the city Suoyang (C*. songaricum* was called "Suoyang" in China) which can be traced back to Tang dynasty in the 7th century. There are two differences between *C. coccineum* and *C. songaricum*: one is the color of the stamens accompanying structure, the other is the length of the tepals. *C. coccineum* is mainly distributed in the Mediterranean, North Africa, Lanzarote, Western Sahara, Somalia, Syria, Israel, Saudi Arabia, Iran and Afghanistan while *C. songaricum* is mainly distributed in Central Asia, Mongolia and China. *Cynomorium spp.* shows zonal distribution which indicates that it spreads continuously. The host range of *Cynomorium* is relatively wide. *C. coccineum* can parasitize plants from the genera *Halimus*, *Salsola*, *Atriplex*, *Arthrocnemum* and *Suaeda* of the Chenopodiaceae, the genus *Limonium* of the Plumbaginaceae, the genus *Inula* of the Asteraceae, *the* genus *Myrtle* of Myrtaceae and the genus *Zygophyllum* of the Zygophyllaceae. *C. songaricum* can parasitize plants from the genus *Nitraria* of the Nitrariaceae and the genera *Peganum* and *Zygophyllum* of the Zygophyllaceae. Judging from the distribution area, *C. coccineum* mainly parasitizes plants from the Chenopodiaceae and *C. songaricum* mainly parasitizes plants from the Nitrariaceae. Our study suggested there were horizontal gene transfers between the common ancestor of *Cynomorium* and plants from the Sapindales. After the divergence of *C. coccineum* and *C. songaricum*, horizontal gene transfer still happened between *C. songaricum* and Nitrariaceae in *atp1* gene. The results indicate that the host choice plays an important role in the divergence of *C.coccineum* and *C. songaricum*. We conducted genetic analysis using ISSR markers on 16 different populations of *C. songaricum* in Northwest China. The results showed that 56.57% of the total genetic variability could be accounted for the differences within populations.

BROOMRAPE POLLINATORS IN THE LIGHT OF FLORAL VOLATILES

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Floral volatile organic compounds (VOCs) are one of the adaptations that plants have evolved to attract and guide pollinators. Despite of their importance almost nothing is known about pollinators in holoparasitic plants from the genera *Orobanche* and *Phelipanche*. The aim of this study was to investigate and compare the composition of pollinator communities associated with broomrapes across large geographical areas and different habitats. Croplands, mountain valleys, subalpine zones, and open, sunny and warm habitats of Slovakia were screened for broomrape pollinators. In parallel we analyzed the floral VOCs emitted by twelve different broomrape species with the aim to identify compounds that play a role in pollinator attraction. VOCs were trapped *in situ* from broomrapes growing in the wild as well as in crop fields of Slovakia using dynamic headspace sampling. The headspace samples were analyzed using GC-MS. Broomrapes were separated into two main groups, a weedy (growing on annual and short lived perennial hosts) and a wild (growing on perennial hosts) group. The composition of the pollinator communities was similar in similar habitats of different regions. On the other hand, weedy species were visited by different pollinators from those that visited wild species. The composition of the pollinator community was significantly richer in wild species, and included social wasps (Vespidae), bumblebees (Apidae), honey bees (Apidae), sweet bees (Halictidae), polyester bees (Colletidae), and hoverflies (Syrphidae). No social wasps, honeybees, bumblebees and hoverflies were recorded on weedy, purple or blue, species. Many of the flower VOCs known to be attractive for bees and wasps are missing in the weedy species. We hypothesise that the loss of pollination in weedy group coincided with a change in floral VOCs. All of these results will be discussed and differences in floral scent chemistry and ecological implications highlighted.

POLLINATION SYSTEMS IN *CYTINUS*: ANTS, RODENTS, ELEPHANT SHREWS, AND MORE

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The enigmatic holoparasitic genus *Cytinus* intrigues with its curiously disjunct distribution, the occurrence of two rare sexual systems, and a unique flower morphology. Most closely related to the Central American genus *Bdallophytum*, *Cytinus* occurs in the Mediterranean (2 monoecious species), South Africa (3 dioecious species), and Madagascar (at least 3 dioecious species). The visible part of these mostly root-parasitic plants consists of one to many-flowered inflorescences composed of radially symmetrical flowers, with 4-9 stiff petals arranged around a central column bearing a ring of anthers or the stigma. At the base of each petal is a nectar chamber. During anthesis, the petals of some species become erect, while in other species the petals remain folded over the central column throughout the flowers' lifespan, thereby providing a mechanism to exclude non-pollinating flower visitors. The reproductive ecology of most *Cytinus* species is unknown. Recently, pollination by ants has been documented in the Mediterranean species *C. hypocistis*, and by rodents and elephant shrews in the recently described *C. visseri*, whose distribution is restricted to high altitude grasslands in South Africa. Flowers of the Western Cape species *Cytinus sanguineus* appear adapted to bird pollination as they are bright scarlet, scentless, and produce copious nectar in open, easily accessible flowers. By contrast, flowers of the other Cape species, *Cytinus capensis,* are dark maroon and remain tightly closed throughout their life. The discovery of a new population of plants tentatively identified as *C. capensis* enabled us to carry out detailed pollination studies in the Groot Winterhoek Wilderness Area in the Western Cape province of South Africa between September and December 2012. Motion-sensor camera recordings revealed visits by nocturnal rodents, which are attracted by the strong, vanilla-like scent of flowers and feed on the nectar. Rodents access the copious nectar by pushing down individual petals along pre-formed hinges, and simultaneously transfer the pollen attached on the fur around their snouts. Insects were not seen visiting the flowers. We compare aspects of the reproductive biology of *Cytinus capensis* with others in the genus, and discuss the evolution of sexual and pollination systems in *Cytinus* based on a well-resolved molecular phylogeny of the genus.

A MOLECULAR TAXONOMY STUDY ON *PHELIPANCHE* SPECIES (OROBANCHACEAE) IN BULGARIA

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Our current knowledge about biodiversity and distribution of genus *Phelipanche* on the Balkans is based mainly on floristic records. According to these records the genus is represented in Bulgaria by 6 species and one putative hybrid. We used a combination of several molecular markers i.e. Inter-simple sequence repeats (ISSR), phytochrom A, RuBisCo large subunit and ribosomal cistron sequences to confirm or update existing data. Specimens of *P. purpurea, P. arenaria, P. mutelii, P. oxyloba*, *P. nana* and *P. ramosa* were collected from different locations in Bulgaria. Total genomic DNA was extracted from fresh or frozen stem tissue and used as template for Polymerase Chain Reaction (PCR*)*. Six previously selected ISSR primers were used to amplify polymorphic microsatellite loci. The amplified unambiguous bands for each primer were scored manually and the result filled in presence/absence matrices that were subjected to cluster analysis. The resulting cladogram, based on the average Euclidean distances, displayed a very clear separation of *P. ramosa* from *P. mutelii.* Two other species - *P. nana* and *P. oxyloba* - also displayed grouping by species. Only samples from *P. purpurea* and *P. arenaria* formed a joint cluster. The data from ribosomal cistron however displayed surprising results. The sequences isolated from *P. arenaria* samples showed 99% similarity with those of *P. purpurea.* This was confirmed by comparison with sequences of *P. arenaria* and *P. purpurea* annotated in NCBI. Previously taxonomists argued that *P. arenaria* in Bulgaria is in fact a variety of *P. purpurea*. Our molecular data confirmed this view. The other interesting finding came from sequences of *P. mutelii*. They were compared with different sequences of *Phelipanche* species, annotated in NCBI. The phylogenic analyses grouped the isolated by us *P. mutelii* sequences with those annotated for *P. rosemarina* (99% similarity), while the NCBI-derived sequences of *P. mutelii* went in another branch of the phylogenic tree.

NATURAL SEED BANK DECLINE OF *PHELIPANCHE MUTELII* IN SOUTH AUSTRALIA

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Successful control, containment or eradication of an annual weed that recruits from seed requires an accurate assessment of seed persistence in the soil seed bank. Quantitative data is lacking for most *Orobanche* or *Phelipanche* species although incidental observations suggest a very long-lived seed bank. We have described the seed bank and quantified the decline in *Phelipanche mutelii* seed viability at three field sites in South Australia. Sampling through the soil profile in cropped and pasture fields demonstrated that the highest proportion of seeds occurs in the top 10 cm of the soil profile. With no recent seed input, seeds are relatively evenly distributed throughout this depth but where broomrape has emerged in the year before sampling, seed is concentrated in the upper 2.5 cm. *P. mutelii* seed banks have a distinct dormancy cycle with peak germination in summer and autumn and dormancy in winter and spring. *P. mutelii* typically germinates in response to autumn rains that follow the dry summers in South Australia. We have ten years of seed burial experiment data for two sites and five years of data for a third site. Seed viability was 90% at the time of burial in sachets constructed from stainless steel or nylon mesh. Burial sites were kept host-free. After ten years at one site on a deep sandy soil, seed viability has declined to 2 % for seed at 10 cm depth and 20% at 5 cm depth. This is in marked contrast to the second site on sandy loam where seed buried in sachets up to 5 cm deep has only declined to 65% viability after 10 years. In our third site also on a sandy loam soil, after five years seed viability at 10 cm depth has declined to 40%. A logistic model fitted to the ten-year data set on deep sand indicates that all seed will have lost viability after 17 years at 10 cm depth and 30 years at 5 cm depth. However, loss of viability at the second site has been too gradual to make any estimates of time to complete loss of viability. In our third site there are sufficient sachets to continue monitoring loss of viability for 30 years. A pot experiment to explore the site differences in viability loss found that soil microbial populations in the three sites are distinct. Further work to identify these micro-organisms is continuing. Soil moisture also hastened the loss of seed viability. Unfortunately the potential extreme longevity of the *P. mutelii* seed bank combined with the continued discovery of further populations of the weed and difficulties of control outside cropped areas has resulted in the cessation of funding for the eradication program for *P. mutelii* in South Australia. Future management will rely on control by individual landholders. Seed longevity estimates are still vital to inform landholders on how long control programs should be in place to prevent future *P. mutelii* incursions.

REPORT OF*CEROPLASTES SP* IN MISTLETOE (*PHORADENDRON BOLLEANUM)* IN SIERRA DE ARTEAGA, COAHUILA, MEXICO

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In Mexico mistletoes occur in more than 1.8 million hectares of forests in several locations. In the Coahuila timber forest resources are scarce and not subject to extraction. These forests are important from the point of view of ecology, scientific and recreational aspects. For this reason we are compelled to protect and preserve them. Due to drought, frost and forest fires, trees have been extremely weak, and susceptible to pest, diseases and parasitic plants. At the Sierra de Arteaga, cypress trees (*Cupressus arizonica*) show an incidence of 71% parasitism by the mistletoe *Phoradendron bolleanum*. In this study we sampled the area which is the junction to "The Tunal" Municipality of Arteaga, Coahuila, to inspect *Cupressus arizonica* trees and we found an uncommon scale insect (*Ceroplastes* spp., Hemiptera: Coccidae). We found that this insect is a new herbivore on mistletoe. *Ceroplastes spp*. was found on 4 of 9 mistletoes parasitizing one tree. This insect could be a good biocontrol agent for controlling the mistletoe *Phoradendron bolleanum.*

ASSESSMENT OF THE LEVEL AND EXTENT OF STRIGA INFESTATION OF CEREAL AND COWPEA FIELDS IN A DRY SAVANNA ECOLOGY OF NORTHERN NIGERIA

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The parasitic weed, *Striga* is a major threat to cereal and cowpea production in the savannas of West and Central Africa. The levels of infestation of Striga are often so high that the crops can suffer 100% yield loss, and farmers are often forced to abandon their fields. In northeast Nigeria, over 85% of fields planted to maize and sorghum and over 60% planted to cowpeas are infested with *Striga* leading to severe yield losses. To plan interventions to manage *Striga* in farmers' fields, there is a need to periodically assess the level and extent of *Striga* infestation in northern Nigeria. Field surveys were undertaken in Jigawa State of northern Nigeria during the 2012 cropping season to assess the level and extent of *Striga* infestation of Striga in cereals and cowpea fields. The soil factors affecting *Striga* infestation were also assessed. The surveys were carried out in 48 communities across 12 Local Government Areas of the State. *S. hermonthica* incidence in sorghum fields in communities ranged from 58 to 100% and in millet from 85-100%. Number of emerged *Striga* plants in sorghum fields ranged from 4,750 – 431,500 plants ha^{-1}. Host reaction ranged from mild firing on 1 or 2 leaves to complete scorching of all leaves with premature death of host plant without head formation. *Striga* population in millet fields varied widely and was in the range of 0 - 251,750 plants ha^{-1}. S*triga gesnerioides* was found in all the cowpea fields surveyed suggesting that this weed is a major threat to cowpea production in the State. The population of *Striga* on cowpeas in the communities ranged from 3,500 – 360,000 plants ha^{-1}. Stepwise multiple regression identified latitude, pH, available P and Cu, and exchangeable K as potentially most important in explaining observed variations in S. *hermonthica* infestation of sorghum. All the variables except available P were positively related to *S. hermonthica* count. The result of the redundancy analysis showed that total N, organic C were negatively correlated with *S. hermonthica* count, the number of *S. hermonthica* attached to sorghum and *Striga* incidence in the study area. For millet Stepwise multiple regression identified soil pH, available P and Zn, and latitude are import variable relating to *Striga* growth. Stepwise multiple regression identified latitude, total N, organic C, exchangeable Ca, ECEC, pH, available Mn and Cu as important variables in explaining observed variations in *S. gesnerioides* population in the cowpea fields.

ECOLOGICAL NICHE DIFFERENCES BETWEEN *RHAMPHICARPA FISTULOSA* AND *STRIGA ASIATICA* IN RAIN-FED RICE

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Rhamphicarpa fistulosa and *Striga asiatica* are parasitic weeds of rain-fed rice in Sub-Saharan Africa. In Kyela, located in the south of Tanzania, both species can be found along the upland-lowland continuum where rice is the dominant crop. To generate weed management recommendations enabling rice farmers to anticipate expected future changes in climate and environment, there is a need to ascertain the environmental niche and plasticity of both species. To that end, ecological ranges of *R. fistulosa* and *S. asiatica* in rice growing environments were investigated. Through field observations parasitic weed species prevalence were linked to soil characteristics and associated weed species. In pot experiments the influence of soil moisture on parasitic weed emergence and performance was examined. In June, 2012, three 3 km transects were installed near rice growing villages in Kyela. Each transect covered 12 rice fields of approximately 1 ha in size, making a total of 36 fields. In each field, three, 1×1m quadrats were evaluated for parasite abundance and weed species associated with each parasitic weed species. Soil samples of 10 cm depth were taken to assess moisture content, texture, organic carbon content (OC), and levels of N, P, K, Mg, Ca, Na, pH, soil electrical conductivity (E.C) and Cationic Exchange Capacity (C.E.C). A pot experiment was conducted twice in Tanzania, using a split-plot design in 5 replicates, with rice infested by either *R. fistulosa* or *S. asiatica*, on the main treatment level, and four water levels at the sub-treatment level: 1) wilting point-field capacity, 2) field capacity 3) field capacity-saturation, and 4) saturation. In the field, *R. fistulosa* was observed in 38 quadrats and *S. asiatica* in 49, while in 21 quadrats no parasitic weeds were noted. A total of 43 associated weed species were encountered. Species exclusively associated with *R. fistulosa* were *Ammannia auriculata*, *Oryza longistaminata, Scleria vogelli, Fimbristylis littoralis* and *Mariscus longibracteatus* while *Spermacoce octodon*, *Pennisetum polystachion, Mitracarpus villosus* and *Rottboellia cochinchinensis* were exclusive to *S. asiatica* habitats. The soils were moderately saline acidic soils with low levels of N, P and OC and high levels of Na, K, Ca and Mg. Soil moisture, silt content, Ca, Mg, Na, EC and CEC were significantly higher in the *R. fistulosa* habitats while relative elevation, and levels of P and K were highest in *S. asiatica* habitats. Non-parasite habitats showed characteristics similar to either *R. fistulosa* or *S. asiatica* habitats but had significantly lower OC, N and K than any of the parasite habitats. The pot experiments showed significant differences in soil moisture preferences between the species. *R. fistulosa* preferred saturated conditions while *S. asiatica* thrived under field capacity conditions, yet each species emerged and survived under all moisture conditions, with the exception of *S. asiatica* under saturated conditions. This revealed that both *R. fistulosa* and *S. asiatica* have markedly different habitat and ecological characteristics and that soil moisture can be a major driver in the expansion of parasitic weeds.

LIMITATION OF CURRENT REPRODUCTION BY RESOURCE AVAILABILITY AND MATING COSTS IN TWO SOUTH AFRICAN *HARVEYA* SPECIES - AN EXPERIMENTAL FIELD STUDY

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Reproduction is the ultimate goal for any organism, but because resources are generally limited, organisms have to commonly trade off competing activities like growth, maintenance, and reproduction. Consequently, reproduction is often submaximal due to resource limitation, and often additionally limited by the availability and quality of mates for offspring production. In plants, resource limitation of current reproduction commonly results in the production of less seeds and fruits than the available number of ovules and flowers. Pollen receipt can limit seed production if received pollen is quantitatively insufficient to fertilize all ovules, and if pollen quality reduces seed production following self-pollination. Loss of opportunities for successful matings that result in viable offspring are summarized under the term "mating costs", and resource limitation and mating costs often interact in limiting plant reproduction. Because of their unusual strategy of resource acquisition from autotrophic hosts, holoparasitic plants are particularly interesting for studies of resource limitation of reproduction. Holoparasite reproduction may be less resource limited than that of autotrophic plants given the direct extraction of concentrated resources from the host, and a body plan largely reduced to reproductive organs. To examine whether current reproduction of *Harveya capensis* and *H. speciosa* is limited by resource availability and/or mating costs, we conducted a field study involving hand-pollinations of all flowers of experimental plants. We saturated stigmas with either outcross- or self-pollen to minimize quantitative pollen limitation, and to determine whether pollen quality affects seed production and fruit set. Pollinator exclusion revealed that both species are incapable of autonomous autogamy, indicating that all natural fruit set is pollinator-mediated. Compared to open-pollinated plants, manual cross-pollination increased fruit set, indicating that reproduction of both species is pollen limited in nature. Self-pollination reduced fruit set, fruit mass, and viable-seed production in both species. Cross-pollination resulted in essentially complete fruit set in both species. Our results provide limited evidence for resource limitation of current reproduction, but indicate that pollinator services and mating costs arising from self-pollination reduce plant fitness. We hypothesize that host quality might determine holoparasite pre-emergence size, precluding detection of resource limitation of reproduction at the flowering or fruiting stage. Overall, the resource demands of perennial parasites should be constrained to enable host survival, possibly also to improve offspring establishment chances. However, given that both *Harveya* species are perennial, it is conceivable that high reproductive investment in one season will affect parasite re-emergence, biomass, and reproduction in subsequent seasons, thus evening out reproductive investment over time.

Session 4: Control and Management

Chair

Jonne Rodenberg and Yaakov Goldwasser

TIMING AS A PARASITIC WEED MANAGEMENT STRATEGY FOR SMALLHOLDER RICE FARMERS

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Farmers in Kyela District, southern Tanzania, consider the parasitic weeds *Striga asiatica* and *Rhamphicarpa fistulosa* as important constraints to rain-fed rice production. Informal surveys among affected farmers revealed their general observation of lower infection levels in early sown crops compared to later sown crops. Increased rainfall irregularities, the recently observed later onset of the rainy season and the frequent occurrence of an early-season drought spell, were however identified as important risk factors to the implementation of early sowing as a management strategy. In staggered sowing date trials, in a *Striga*-infested upland and a nearby *Rhamphicarpa* -infested lowland farmers' field, carried out in two consecutive cropping seasons (2012-2013), we tested the farmers' hypothesis that early sowing reduces parasitic weed infection levels in rice. To investigate whether the aforementioned risks of early sowing could be alleviated we included cultivars with different cycle lengths (but comparable parasitic weed resistance levels) in this trial; the traditional long-duration cultivars that farmers grow in this region (Kilombero, in both trials, and Mwangulu in the *Striga* trial) as well as modern cultivars with shorter crop cycles (NERICA-14 in the *Striga*-trial, NERICA-L-20 and IR64 in the *Rhamphicarpa*-trial). Each trial consisted of four (2012) and five (2013) sowing dates with 2-week intervals and three rice cultivars in five replicates. Rice performance and parasitic weed infection levels and reproductive success were monitored and farmers were asked to evaluate and rank the treatments.

Striga numbers and seed capsule production reduced with each 2-week delay in sowing date; however rice yields of the first sowing dates were significantly higher than that of the two latest sowing dates for all cultivars. This is likely the result of a shortening of the growing season for the late sown crops. Farmers seemed to understand this as they indicated a preference for the intermediate sowing dates and the early maturing NERICA-14. *Rhamphicarpa* numbers and seed capsule production were only significantly higher in rice sown at the latest sowing date, while rice yields were significantly reduced with each later sowing date. At the earliest sowing date, the early maturing NERICA-L-20 produced a significantly higher grain yield than the other cultivars. While farmers had no clear preference for any rice cultivar in the *Rhamphicarpa* trial, they ranked the sowing dates in decreasing chronological order, with the highest preference for the earliest date. Hence, while *Striga* numbers, and particularly *Striga* seed capsule production, reduced progressively with each 2-week delay in sowing, the *Rhamphicarpa* numbers and seed capsule production increased by sowing later. Cultivar cycle lengths had less clear effects but early maturing cultivars are likely to suffer less from parasitism because of the shorter infection times while parasitic weeds may benefit shorter from host resources for seed production. By adjusting the timing of their crop to the rice growing environment and the prevalent parasitic weed species, farmers can reduce parasitic weed infection levels and reproduction and optimize their crop yields. This adds to the basket of options for resource-poor farmers working in parasitic weed infested areas.

THE ROLE OF RESISTANT RICE VARIETIES IN A LOCALLY ADAPTED INTEGRATED *STRIGA* MANAGEMENT APPROACH

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The parasitic plant *Striga asiatica* (L.) Kuntze is a common weed in the Mid-West of Madagascar and is particularly problematic in the high altitude (800-1,300 m a.s.l.) rain-fed upland rice-maize rotation systems where it causes serious yield losses. Soils are characterized by low organic matter contents and high degradation (soil erosion) risks. A 3-year integrated *Striga* management trial was initiated in the 2011/2012 cropping season, to compare the conventional mono-crop rice – maize rotation practice, involving seasonal tillage and crop residue removal (S1), with three rice – maize rotation systems, combining zero-tillage and annual or perennial cover crops: a rotation with rice and maize intercropped with a *Vigna unguiculata* - *Mucuna* spp. relay-crop (S2), a rotation with rice and maize intercropped with *Vigna umbellata* (S3), and a rotation with rice and maize, both intercropped with the perennial cover crop *Stylosanthes guianensis* (S4). Crop residues in S2-S4 were not removed. Three rice varieties were tested alongside: the locally popular, but *Striga* susceptible B22, the locally adopted *Striga* resistant NERICA-4, and the newly released and moderately *Striga* resistant NERICA-9. The hypotheses are that combining zero-tillage with intercropping will reduce the *Striga* infestation rates in rice and maize, while protecting the soil and improving the organic matter content. The use of *Striga* resistant rice varieties should enhance *Striga* suppression in these systems.

In season-1, the mean *Striga* densities on rice variety B22 (at harvest) was 8 times higher than on NERICA-9 and 18 times higher than on NERICA-4 ($P<0.05$). In season-2, the mean *Striga* density on B22 (at 75 days after sowing; DAS) was 5.3 times higher than on NERICA-9 and 19.3 times higher than on NERICA-4. Moreover, a spectacular carry-over effect of this *Striga* resistance from season-1 to season-2 was observed; where maize followed rice variety B22, the mean *Striga* density (at 70 DAS) was almost double that of maize grown after NERICA-9 and 21 times higher than in maize following NERICA- 4. In season 1, mean *Striga* density (at harvest) in the rice mono-crop plots was nearly two times higher than in the rice – *Stylosanthes* plots ($P<0.05$). *Striga* density in maize was too low to identify significant differences between crop situations. In season-2, mean *Striga* densities (at 75 DAS) in maize S1 and maize S3 were significantly higher than densities in maize S4 (no *Striga*) and maize S2. *Striga* densities in rice S1 and rice S2 were significantly higher ($P<0.05$) than in rice S3 and rice S4.

The use of *Striga* resistant rice varieties significantly reduces *Striga* densities in the current as well as in the following crop. *Striga* densities are highest in a mono-crop rice – maize rotation system with conventional tillage and lowest in a rotation system with rice – *Stylosanthes* -intercrop – maize – *Stylosanthes* - intercrop at zero-tillage. The latter system, with continuous soil coverage, is most promising for resource-poor farmers of the *Striga*-infested and degradable highlands of Mid-West Madagascar.

PHELIPANCHE AEGYPTIACA CONTROL IN TOMATO BY APPLICATION OF IMAZAPIC THROUGH DRIP IRRIGATION

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Drip chemigation of imazapic for *Phelipanche aegyptiaca* control in tomato has been tested in Israel but has yielded inconsistent results. In recent studies we examined imazapic movement in water and soil in pot and field experiments. The aim of this study was to test the efficacy of drip chemigation of the herbicide on *P. aegyptiaca* control. We tested the susceptibility of *P. aegyptiaca* to drench-applied imazapic at all parasite growth stages: seed and seedling in Petri dish, attached to tomato host plants in PE bags, attached to tomato host plants in pots and finally under field conditions. In the pot and field studies parasite and host plant growth and yield was monitored, accompanied by LC/MSMS analysis of soil and plant imazapic concentrations. Preconditioned seeds and seedlings in Petri dish were not affected even at 5000 ppb imazapic. The herbicide killed only parasite seedlings following their attachment to tomato host roots and at concentrations starting at 4 ppb in the lab and in field conditions. We found that the movement of imazapic in the soil is limited and its $t_{1/2}$ value under field conditions was 7-9 days, thus new *P. aegyptiaca* shoots emerged in the field starting at 3 weeks after imazapic application. Concentrations higher than 5 ppb controlled the parasite but were toxic to the tomato host plant. An experiment in which tomato plant roots were split into 2 pots revealed that the herbicide is not transported from treated roots to untreated roots. Thus for efficient control we have to insure the herbicide is delivered, distributed, and present in the toxic concentration throughout the entire tomato root system, from the time of parasite establishment up to crop maturation. The analysis of imazapic in tomato foliage tissues following the drip chemigation treatment is underway and will be added to soil, water and herbicide data. All data will be incorporated into the HYDRUS 2D/3D numerical model for computing and validating the optimal herbicide chemigation variables ensuring efficient *P. aegyptiaca* control.

EGYPTIAN BROOMRAPE CONTROL IN TOMATO WITH MALEIC HYDRAZIDE

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One of the most destructive pests in agriculture is the broomrapes (*Phelipanche* and *Orobanche* species*),* which is a limiting factor in the production of many crops, including processing tomatoes. Broomrape can be effectively controlled in tomato by three successive applications of sulfosulfuron at a rate of 37.5 gr a.i. ha^{-1} followed by upper irrigation of 300m^3 ha^{-1}. In addition imazapic at a rate of 7 gr a.i. ha^{-1} should be applied twice toward the end of the growing season. The method was registered for use in Israel but most tomato growers in the country are not using it because its expensive, laborious, requires special equipment (moving pivot) and above all, the long residual effect of sulfosulfuron in the soil interrupts normal crop rotation. Maleic hydrazide (MH) is a plant growth regulator (sprout inhibitor) and herbicide that acts by inhibiting cell division in plants. It is used to control sprouting of potatoes and onions, suckers in tobacco, and growth of weeds, grasses and trees in/along lawns, turf, ornamental plants, non-bearing citrus, utility and highway rights-of-way, airports and industrial land. In the last 3 years we conducted field experiments to control broomrape with MH. Application through drip irrigation was unsuccessful but 4-5 foliage applications 14-21 days apart at a rate of 30-54 gr a.i. ha^{-1} starting at 200 growth degree days (GDD) completely controlled the parasite producing high tomato yield. However, under very high broomrape infestations, while broomrape control was still excellent, the yield was low and did not differ from the yield in the control plots. Advancing the first MH application to 100 GDD resulted in normal high yield with excellent broomrape control. No phytotoxic effects or yield reduction could be observed in field experiments conducted in broomrape-free fields.

EFFECTS OF *DESMODIUM* ROOT EXUDATES ON *PHELIPANCHE RAMOSA* AND *OROBANCHE CRENATA* AND THEIR ASSOCIATED HOSTS

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Root parasitic plants such as *Striga, Phelipanche* and *Orobanche* species cause severe loss of crop production worldwide. In some areas of East Africa, intercropping with *Desmodium* species has given good control of *S. hermonthica*. The root exudates of *Desmodium* contain compounds that affect germination, early development and attachment of *S. hermonthica* to the host plants. Do these effects of *Desmodium* on *Striga* also apply to *Phelipanche* and *Orobanche* species? And, what are the implications for the use of *Desmodium* for control of parasitic weeds other than *Striga*?

Significant reductions in infestations by these parasites were obtained when their respective hosts, tomato and pea, were planted in soil moistened with water draining from pots where *D. uncinatum* and *D. intortum* plants were being grown. Furthermore, exposure to *Desmodium* root exudates for 12 rather than four weeks was more effective in controlling the parasites. Exudation of both germination stimulants and attachment inhibitors was, however, significantly affected temperature.

However, a severe and concurrent reduction in the growth and yield of all host crop plants was also observed. When exposure to *Desmodium* was removed, host plants showed some recovery and improvement in growth and yield. Furthermore, the higher the concentration of *Desmodium* roots exudates, the more detrimental the effect on the growth and yield of host plants.

Using liquid chromatography-mass spectrometry and nuclear magnetic resonance techniques, potential active compounds included epicatechin, catechin, quercetin and rutin as inhibitors of crop growth and isoschaftoside, uncinanone A, uncinanone B and uncinanone C as influencing parasite germination and attachment. Practical implications of these results for parasite weed management will be discussed.

SHOOT THE MISTLETOE - A NEW METHOD FOR CONTROLLING MISTLETOES IN TREES

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Mistletoes are an important silviculture problem in many countries, causing growth problems in urban trees as well as big losses in lumber, pulp or fruit harvests. Some of the most hazardous mistletoes such as *Viscum album* (North America and Europe), *Arceuthobium globosum* (North America), *Psittacanthus schiedeanus* (Mexico), *Phoradendron crassifolium* (Brazil) and *Tripodanthus acutifolius* (Brazil) are very widespread in our planet, infesting many useful plants. One of the most difficult challenges in controlling the mistletoes is to reach them when they are attached to high branches. Another problem is the difficulty in removing them without damaging unnecessarily the tree architecture by cutting too many or too important parts of the canopy. Anyhow, the manual removal of mistletoes is an expensive and difficult process, also risky for operators. So we had the idea to develop a surgical attack using a "prolonged release" approach. To this purpose, we created an adhesive pellet, capable to attach to the parasitic body and kill it slowly but surely, without damaging the host. The developed pellet would be loaded with specific herbicide compositions able to kill only the parasite, without causing major effects to the hosts. The general idea was to use paintball capsules loaded with the killing agent, thrown away with paintball-like compressed air shotgun. The challenges we faced were: 1) to reach the canopy with precision; 2) to find a way to see which parasite was hit; 3) to create a pellet non attractive to animals; 4) to obtain good adherence to branches, even under harsh climate conditions; 5) to insulate the killing agent, so it would not escape to the atmosphere. Therefore, we had to develop a special encapsulating material for the killing agent and a designed device for delivering it. At this moment new air propelled applicators are being designed to deliver our pellets. Killing agent, applicators and encapsulating agent, are protected by new patents and we hope to bring them to the market pretty soon.

USE OF POTENTIAL NON-HOST CROP GENOTYPES AND ALLELOPATHY PROPERTIES OF LOCAL PLANTS FOR CONTROLLING *STRIGA HERMONTHICA* IN BURKINA FASO

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Striga hermonthica (Del.) Benth. is a major biotic constraint to cereal productions in sub-Saharan Africa. The genotypic influence in stimulating *Striga* germination from seven non-hosts was evaluated as trap crops in a bio-assay using the root-cut technique. Nine cotton genotypes, 15 cowpea genotypes, 6 groundnut genotypes, 5 sesame genotypes, 4 Bambara nut genotypes, 2 soybean genotypes and a rice bean genotype were compared to *Striga*-susceptible sorghum S29 (control) to identify those endowed with a potential capacity to induce suicidal germination of *S. hermonthica* seeds. The 10% aqueous extracts of eight local plants: *Acanthospermum hispidum* de Candolle, *Cassia obtusifolia* L., *Eucalyptus camaldulensis* (Dehenhardt), *Faidherbia albida* (Del.) A. Chev., *Lippia multiflora* (Moldenke), *Stereospermum kunthianum* Cham., *Tridax procumbens* L. and *Vitellaria paradoxa* C. F. Gaertn, were also screened in a bio-assay to evaluate their allelopathic properties to induce or inhibit *Striga* seed germination elicited by GR 24. The genotypes of the non-host trap crops exhibited significant differences in their ability to stimulate the germination of *Striga*. *S. hermonthica* seed germination rates of at least 75% were recorded for nine cotton genotypes. *Striga* germination rates induced by both cowpea and groundnut genotypes were lower compared to that of *Striga*-susceptible sorghum S29 (80%). Sesame genotypes induced *Striga* germination rates ranging from 16.1% to 27.3%, and the variety S42 led to the greatest rate. Among soybean genotypes, G.196 and G.197 significantly induced *Striga* germination with 11.9% and 24.4%, respectively, while Bambara nut genotypes KVS-075 and KVS-143 gave the highest rates of *Striga* germination. The rice bean genotype led to *Striga* germination rate of 6.6%. The 10% aqueous extract from *E. camaldulensis (*roots) and *L. multiflora (*leaves) significantly reduced *Striga* seed germination with 86.3% and 46.5% inhibition rates, respectively. The 10% aqueous extracts of all plant species stimulated *Striga* seed germination. The most effective in stimulating *Striga* seeds were the 10% aqueous extracts from *E. camaldulensis* (leaves) and *F. albida* (bark) leading to *Striga* germination rates more than 50%. The 10% aqueous extracts from the four other ones significantly stimulated *Striga* germination and the rates varied between 25.2% and 48.1%. The prolonged use of non-host plants that produced stimulants or inhibitors of *S. hermonthica* germination may reduce or inactivate its seed-bank in the soil, respectively. Non-host crop genotypes that induced *S. hermonthica* seed germination rates of at least 10% may be recommended for use in cropping systems like rotation or intercropping with cereals, particularly in an integrated management approach against *S. hermonthica.* The results suggested that local plant products may be used in controlling *Striga* as bio-herbicides and would also be a safe alternative approach.

COMBATING PURPLE WITCHWEED (*STRIGA HERMONTHICA* (DEL.) BENTH.) WITH ACETOLACTATE SYNTHASE-MODIFIED MAIZE SEEDS IN THE WEST AFRICAN SAVANNAS

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The purple witchweed, *Striga hermonthica* (Del.) Benth. is a serious threat to cereal production in the savannas of west and central Africa and causes up to 100% yield losses in susceptible maize varieties. A recent approach to control *S. hermonthica* is seed treatment with low doses of acetolactate synthase (ALS)-inhibiting herbicides, such as imazapyr. These herbicides are phytotoxic, therefore maize cultivars targeted for seed treatment should possess a resistance gene to them. A field trial was conducted in four locations (Abuja, Mokwa, Zaria, and Sabongari) in Nigeria in 2007, to evaluate the performance of imazapyr coated and uncoated seeds of experimental hybrids that combine the imazapyr resistance (IR) gene with polygenic resistance to *S. hermonthica* under infestation. Treatments were eight IR-maize hybrids and three checks without the IR gene (commercial, *Striga* tolerant, and *Striga* susceptible hybrids). Averaged across all locations, the coated IR hybrids yielded 18% more than the uncoated IR hybrids and supported fewer *S. hermonthica* plants. The IR hybrids were competitive with the commercial hybrid check in yield potential when they were not coated with the herbicide. The IR hybrids yielded 2226 to 3574 kg/ha and 2056 to 2997 kg/ha of grain with and without seed herbicide coating, respectively. The susceptible hybrid check had severe damage from *S. hermonthica* and a yield loss of 93% under infestation without herbicide coating. The findings indicate that ALS-modified maize seeds with resistance to *Striga* were effective for *S. hermonthica* control both with and without herbicide coating.

SCREENING EFFECTS OF CRUDE AQUEOUS SAWDUST EXTRACTS ON GERMINATION OF *STRIGA HERMONTHICA* SEEDS

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Striga hermonthica infestation of cereal crops is one of the major constrains plaguing subsistent farmers in sub-saharan Africa. Approaches towards implementing an effective *Striga* control strategy often targets three critical phases of its life cycle: the pre-emergent phase that include the germination, the haustorial attachment stages and the post-emergent phase. In this study, the effect of crude aqueous sawdust extracts (prepared by dissolving 100 g of sawdust in 100 ml of water) on the germination of *S. hermonthica* seeds was investigated. *Striga* seeds were exposed to the sawdust extracts during the moist preconditioning stage prior to exposure to the germination stimulant, GR24. Preconditioning of *Striga* seeds was performed in petri-dishes containing extracts from 14 sources (treatments). Eleven of the treatments were from different sawdust types while the remaining three (sterile distilled water, river-sand, and top-soil) were designated as control treatments. The screened sawdust sources were: *Pycnanthus angolensis,* Myristicaceae (Acom); *Terminalia superba,* Combretaceae (White Afara); *Bombax buonopezense*, Malvaceae (Bombax), *Nesogordiana papaverifera*, Malvaceae (Danta); *Tieghemella heckelii,* Sapotaceae (Guruba macuri); *Pausinystsalia macroceras,* Rubiaceae (Nikiba); *Celtis adolfi-trideria,* Ulmaceae (Ohia); *Ricinodendron hendelotii,* Euphorbiaceae (Okhwen); *Nauclea diderrichii,* Rubiaceae, (Opepe); *Trilepisium madagascariense,*Moraceae, (Ukputu); *Ficus exasperate,* Moraceae (Ukwen). Results showed significantly lower (55-75% less) *Striga* seed germination in five of the sawdust treatments compared to other treatments including all of the controls. Among the five sawdust sources the order from highest to least effect on reduction of *Striga hermonthica* seed germination was: Ukumen > Afara > Ohia > Bomba > Acom. Crude aqueous sawdust extracts from Ukumen, Afara, Ohia, Bomba, and Acom have potential to reduce optimal germination of *S. hermonthica* seeds and may be useful in controlling *Striga* infestation in crop fields.

MANAGEMENT OF *STRIGA HERMONTHICA* IN MAIZE WITH NITROGEN FERTILIZATION AND INTERCROPPING WITH JOINTVETCH (*AESCHYNOMENE HISTRIX*)

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The parasitic plant witchweed (*Striga hermonthica*) belongs to the family Orobanchaceae that can cause up to 100% crop grain yield loss in cereals. A study was conducted at Minna, Nigeria in 2011 and 2012 with the aim of reducing the effect of *S. hermonthica* on maize and hence improve its grain yield by application of 0, 60, 90 and 120 kg N ha^{-1} to maize or inter-planting it with Jointvetch (*Aeschynomene histrix*) alternately or same hill. The treatments were arranged in a randomized complete block design and replicated three times. The maize was sown on a land heavily naturally infested with *S. hermonthica.* The results showed that maize with 0 kg N ha^{-1} had the least number of plant stand and highest *Striga* shoot density at 12 weeks after sowing (WAS) which culminated in lowest maize grain yield. The alternate inter-planting of maize with *A. histrix* reduced *Striga* shoot density per plot by 49.0%, 26.6%, 13.6%, 41.0% and 18.8% compared with 0, 60, 90 and 120 Kg N ha^{-1} application and same hill inter-planting, respectively. It is concluded that alternate inter-planting of maize with *A. histrix* consistently had the highest maize grain yield in the two years which was on average 64.2%, 39.4%, 39.0% and 37.0% higher than 0, 60, 90 and 120 Kg N treatments, respectively.

REACTIONS OF DIFFERENT GENOTYPES OF MAIZE TREATED WITH VARYING RATES OF IMAZAPYR IN YOLA NIGERIA

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Many families in Nigeria depend on maize as their source of staple food. In recent years however, maize productivity has declined. One of the major causes of declining maize yield in Nigeria is Striga hermonthica menace. Research efforts have led to the discovery of the treatment of maize seeds with imazapyr herbicide as a means of controlling striga in IR maize. This technology has great potentials for success in the control of striga in maize. It has however been observed that from the 12th week after sowing (WAS), striga plants do emerge and cause burning symptoms in some genotypes. This shows that the level of resistance may vary with varieties and perhaps the rate of imazapyr applied. Therefore, field experiments were carried out in Yola, Nigeria to determine the effects of eight rates of imazapyr (0, 15, 30, 45, 60, 75, 90 and 105 g/ha) tested against twelve maize genotypes on striga effects on maize. The treatments were arranged factorially in a randomized complete block design. Maize seeds were primed in different rates of imazapyr, dried under shade and planted. About 5000 striga seeds were applied per maize stand one week after maize emergence. The result shows that there were no significant differences among imazapyr rates on striga emergence at 10 and 12 WAS but all the rates significantly differed from the control. Striga emergence was significantly affected by maize genotypes with striga counts of <1 to 28 strga plants per plot at 12 WAS compared to the control that had 1780 striga plants/plot. This implies that some genotypes are capable of maintain striga free plots from planting to maturity. The highest grain yield of 7 t/ha was recorded in the 15 g/ha of imazapyr while 105 g/ha of imazapyr yielded 4.2 t/ha as against the control that had 0.85 t/ha. Similarly, grain yield varied with maize genotypes with the highest grain yield of 7 t/ha compared to the control where 0.89 t/ha was obtained. This shows that even under high striga infestation, reasonable maize yields are possible with imazapyr application.

STUDIES ON THE POTENTIAL OF NEEM TREE PRODUCTS AS BIOAGENTS FOR MANAGEMENT OF *STRIGA HERMONTHICA* IN MAIZE

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Experiments were conducted in the laboratory, pot and field to study the potential of neem tree products as bioagents for the management of *Striga hermonthica* in maize. In the laboratory, the effect of cut-roots of maize wetted with five different concentrations (0, 25, 50, and 100) of either neem leaf or neem bark extract on seed germination in *Striga hermonthica* were studied. Neem leaf/bark extracts were prepared by soaking 200 g of leaf/bark powder in 1 L of water. After decantation, the extracts (100%) were diluted with water to make 25 to 75% extract solutions. Cut roots of maize and distilled water served as the positive and negative controls, respectively. The design of the experiment was set up in Completely Randomized Design with four replications. During the rainy season of the year 2011, potted experiments were carried out to evaluate the effect of neem tree products on *Striga* infestation in maize. Two separate experiments were carried out, each consisting of three maize varieties and four treatments of neem (neem bark, neem leaf, neem fruit and no neem) powders at the rate of 200 g neem powder per 1 kg maize. The design of the experiments was Randomised Complete Block Design with three replications. In the next cropping (rainy) season i.e. year 2012, field experiments were conducted on naturally *Striga*-infested site, as a follow up to the potted experiments to study/ verify the effect of neem tree products as potential bioagents for the management of *Striga* and other weeds in maize. Three maize varieties were again used with neem leaf or bark powder incorporated into the soil and used as seed dressing on maize. The results of the laboratory assay showed that maize cut roots (positive control) stimulated *Striga* seed germination by 43%, whereas the negative control (water) did not trigger germination of *Striga s*eeds. With the addition of neem, *Striga hermonthica* seed germination was reduced to 21% and 14% in 25% and 50% neem leaf extract, respectively. When the concentration of neem leaf/bark extract was increased to 75% or 100%, *Striga* seeds failed to germinate. Neem bark extract was more effective in suppressing *Striga* seed germination than neem leaf extract. Results of the potted experiments showed that maize seeds dressed with either neem leaf or bark powder, emerged, silked and matured earlier than control (no- neem). In pots where neem bark was applied on maize variety Oba 98*, Striga* emerged and flowered earlier compared to the local maize variety. Application of neem bark powder reduced *Striga* seedling emergence from 11 plants/pot to 7. In the field experiments, the type of treatment used had significant difference on the number of days to weed emergence and dry weight of maize at maturity. Neem bark powder delayed the emergence of weeds most, followed by neem leaf powder as compared to the plots where neem was not applied. A few *Striga* seedlings emerged in scattered pattern on the experimental site. Therefore, a meaningful result could not be obtained. However, *Striga* and other weeds, which were observed prior to the commencement of the experiment, did not emerge after neem treatment. This indicated that the application of neem tree products had some control over *Striga* and other weeds on the site.

ISOLATION OF FUNGI INFECTING MISTLETOE *PHORADENDRON MACROPHYLLUM,* AT SALTILLO, MEXICO

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The mistletoe *Phoradendron macrophyllum* parasitizes cultivated pecan trees (*Carya illinoinensis*) in the area of Saltillo, Mexico. A bioassay was performed to determine and evaluate the fungi associated with this parasitic plant as a future biocontrol agent. Mistletoes were collected from pecan trees in Saltillo. In the Laboratory of Phytopathology, mistletoe leaves were washed with tap water, disinfected with sodium hypochlorite 3%, rinsed with sterile distilled water and plated in culture medium (potato Dextrose Agar). Samples were incubated at 25°C for 7 days. After morphological identification fungi were mass-produced. The following fungi were identified: *Fusarium oxyosporum, Alternaria alternata* and *Alternaria infectoria.* Pathogenicity was determined using mistletoe leaves in a humid chamber (moist filter paper) in Petri dishes; an agar culture explant (50 mm in diameter) was placed on the paper and a leaf was placed on top of the explant. Tissue response (necrotic lesion) was measured with vernier (mm) after 72 hours until the fungus invaded the entire leaf of mistletoe. The evaluation was done with a completely randomized design with factorial arrangement 4 X 17 with 7 replications per treatment, the number the expof treatments was based on the number of fungi. These data were evaluated with the SAS statistical program 2004. The analysis of variance (ANOVA) had a coefficient of variation of 50.92%. *Fusarium oxyosporum* in 17 days invaded the leaf. Means of necrosis in cm for each fungus were: *Fusarium*, *oxyosporum* 0.862, *Alternaria alternata* 0.523, Alternaria *infectoria* 0.517 and control 0.433. The significance level used in multiple comparison tests between means was 0.001, and a least significant difference of 0.0994. *Fusarium oxysporum* is the mistletoe fungus that attacks more aggressively the leaves in this bioassay.

Session 5: Crop Resistance and Tolerance

Chairs

Mike Timko and Julie Scholes

IDENTIFICATION OF GENES CONTROLLING COMPATIBLE AND INCOMPATIBLE INTERACTIONS OF COWPEA WITH *STRIGA GESNERIOIDES*

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Cowpea, *Vigna unguiculata* L. Walp., is one of the most important food and forage legumes in the semi-arid tropics. While most domesticated forms of cowpea are susceptible to the root parasitic weed *Striga gesnerioides*, several cultivars have been identified that show race-specific resistance. Cowpea cultivar B301 contains the *RSG3-301* gene that confers resistance to *S. gesnerioides* race SG3, but is susceptible to race SG4z. When challenged by SG3, roots of cultivar B301 develop a hypersensitive reaction (HR) at the site of parasite attachment. This is followed by subsequent browning, and death of the attached parasite. Analysis of gene expression in the roots of the cowpea cultivar B301 during compatible (susceptible) and incompatible (resistant) interactions with *S. gesnerioides* races SG4z and SG3, respectively, using a Nimblegen custom design cowpea microarray identified distinct changes in global gene expression during successful and unsuccessful *Striga* parasitism. We found that accompanying the visible hypersensitive response is the up-regulation of genes involved in signal transduction and biosynthetic processes associated with formation barriers to prevent parasite ingress (e.g., cell wall biogenesis and lignification), pathways involved in response to oxidative stress, signaling pathways for secondary metabolism, and pathways involved in multiple plants' biosynthetic and chemical detoxification processes. In contrast, B301 roots successfully parasitized by SG4z showed no phenotypic manifestation during parasite ingress through the cortex but alterations in gene expression in pathways associated with cellular differentiation and growth, cell signaling and metabolism, and defense signaling. In particular, there was dramatic down-regulation of gene expression involved in auxin transport and signaling, both critical for cellular growth and proliferation, and down-regulation of the expression of genes involved in cell wall growth (e.g., expansins) and reinforcement (e.g., enzymes of cellulose, lignin, and callused formation). Specific defense related genes and pathways could be defined as unique to the resistance mechanism. In addition, some genes and pathways up-regulated in the host resistance response to SG3 were found to be repressed in the susceptible interactions, suggesting that the parasite is targeting specific components of the host's defense. To further elucidate the role of candidate genes involved in the resistance response, a composite plant system was developed for cowpea which allowed the selective silencing of gene targets in B301 roots. These analyses identified several novel components of the resistance mechanism to *Striga* including both transcriptional regulators and cell structural components. The role of these components will be discussed in terms of their broader implications to other *Striga*-host associations and mechanisms for targeted amplification of resistance of crops to parasitic weeds.

BREEDING FOR *STRIGA* RESISTANCE IN PEARL MILLET: RESPONSE TO FIVE CYCLES OF RECURRENT SELECTION

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Pearl millet [*Cenchrus americanus* (L.) Morrone, comb. nov.] is the staple food crop of millions of poor rural families in Africa and parts of India, and so plays a critical role in food security. In sub-Saharan Western and Central African regions, *Striga hermonthica* (Del.) Benth remains a major persistent biotic threat to pearl millet production. Yield losses due to *Striga* parasitism can attain 100% in susceptible cultivars, and the most severely affected are subsistence farmers. Host plant resistance to *Striga* is widely recognized as an important component of long-term integrated *Striga* control. Since 2006, the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), in partnership with the national agricultural research institute in Mali (*Institut d'Economie Rural, IER*), has developed a diversified *Striga*-resistant pearl millet genepool. In total, this genepool was subjected to five cycles of recurrent selection targeting mainly *Striga* resistance and panicle yield, and to a lesser extent downy mildew resistance. To assess selection progress, two-hundred full-sib families (FS) representing the C_5 selection cycle were evaluated together with the genepool parental landraces, experimental varieties derived from previous cycles and local checks in *Striga*-infested fields during the 2011 rainy season at Sadoré (Niger) and Cinzana (Mali). According to our knowledge, this is the first report describing effects of recurrent selection for quantitative *Striga* resistance in pearl millet. *Striga* resistance, downy mildew resistance and panicle yield were significantly improved by recurrent selection. The accumulated percentage gain from selection amounted to 51 – 1% lower *Striga* infestation (measured by area under *Striga* number progress curve, ASNPC), 46 – 62% lower downy mildew incidence, and 49 – 31% higher panicle yield of the C_5-FS compared to the mean of the genepool parents at Sadoré/Cinzana, respectively. Experimental varieties selected from previous cycles also revealed lower ASNPC and mostly higher yield compared to genepool parents at their selection sites. Significant genetic variation among the C_5-FS and operative heritabilities of 76% (Cinzana), 84% (Sadoré) and 34% (combined across locations) for ASNPC will enable continued selection gain for *Striga* resistance. The observed highly significant genotype by environment interaction variance underlines the importance of site-specific extraction of new experimental varieties from the genepool. The C_5 broad-based genepool can continue to serve as breeding population for further selection in collaborative pearl millet improvement programs and for extraction of new experimental varieties with specific adaptation to different target sites. Such cultivars with quantitative resistance may play an important role in integrated *Striga* control in pearl millet in West Africa.

NATURAL VARIATION IN RESISTANCE AGAINST PARASITIC PLANTS

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Root parasitic plants of the *Orobanche* and *Phelipanche* genus (broomrapes) are among the most damaging agricultural weeds in the world. They do not have chlorophyll or functional roots and completely rely on their host for the acquisition of assimilates, nutrients and water. Breeding for parasitic plant resistance has been only marginally successful, also because selection was usually done for field resistance without establishing the resistance mechanism involved. However, there is ample evidence in the literature that resistance can depend on several different mechanisms, such as the induction of low germination, the presence of growth inhibiting metabolites in the exudate, the inability of the parasite to make a haustorium and the hypersenstive response. In this project we explored the natural variation in resistance and resistance mechanisms against the parasitic weed *P. ramosa* (branched broomrape) in *Arabidopsis thaliana*, and used this information to perform a genome wide association study. For this, a largely unstructured core set consisting of 349 Arabidopsis ecotypes was screened covering over 80% of the genotypic variation present within this species. Arabidopsis seedlings were grown in rhizotron petri dish systems and pre-germinated *P.ramosa* seeds were spread along the host roots. Photos were taken every two weeks in order to monitor the development of the attachments. Numbers of pre-germinated seeds that were in close vicinity to the roots and numbers of tubercles and spiders were counted. Furthermore, diameters of tubercles and spiders were measured using ImageJ. Genome-wide association mapping was performed and significant SNPs were identified for different resistance mechanisms such as low attachment capacity and reduced developmental rate. The results will finally be confirmed and the information resulting from this study will be translated to the commercial crop tomato.

VARIATION IN RESPONSE OF A RESISTANT SUNFLOWER CULTIVAR TO *PHELIPANCHE AEGYPTIACA* AND *OROBANCHE CUMANA*

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The broomrapes (*Orobanche* and *Phelipanche* spp.) are obligatory chlorophyll-lacking root parasites, which parasitize broad leaf vegetables and field crops worldwide. Confectionary sunflower is an important economical crop that is parasitized by *O. cumana* and *P. aegyptiaca*. High infestation level can cause total yield losses. Growing sunflower in a crop rotation that includes other *P. aegyptiaca* hosts e.g. chickpea, vetch, potato and tomato enriched the seed-bank of the soil with broomrape seeds. Sunflower cultivars grown for oil production exhibiting high level of resistance to broomrapes were introduced in Europe during the last decades. Similarly, several confectionary sunflower varieties that were introduced in Israel exhibited high level of resistance to *O. cumana* and to *P. aegyptiaca*. Recently, a new resistant confectionary sunflower cultivar 'Emeq 3' was developed based on a trait taken from a Spanish oil sunflower. The objective of the current study was to characterize the variation in the response of 'Emeq 3' to *P. aegyptiaca* and *O. cumana.* Resistant sunflower 'Emeq 3' and sensitive sunflower D.Y.3 (Shaar Ha'amaqim, Israel), were grown in polyethylene bags (PEB) under temperature controlled conditions. The PEB were artificially infested with seeds of *O. cumana* or *P. aegyptiaca,* monitored on a weekly basis and imaged using a stereoscopic binocular. Parasitism dynamics for germination, attachments and tubercles production were monitored for both sunflower varieties for each broomrape species. Seeds of two broomrape species germinated 21-28 days after planting, at the same rate for both sunflower varieties. The sensitive sunflower D.Y.3, was equally sensitive to both parasites - *P. aegyptiaca* and *O. cumana* in terms of attachment number and tubercle production. However, 'Emek 3' was absolutely resistant to *O. cumana* and sensitive to *P. aegyptiaca*. The incompatibility between 'Emek 3' and *O. cumana* was expressed at the attachment and penetration stage. It was clearly indicated that *O. cumana* seedlings failed to penetrate into the resistant 'Emek 3' sunflower roots. It could be postulated that 'Emek 3' sunflower that was bred for resistance to *O. cumana* in Spain is resistant only to *O. cumana* in Israel but not to *P. aegyptiaca*. Interestingly, no such differences in response to *O*. *cumana* and *P*. *aegyptiaca* were reported earlier in a local resistant sunflower cultivars 'Ambar' in Israel. Field studies are currently in progress to validate the resistance response observed in the laboratory. Further study will be conducted to confirm the resistance mechanism of 'Emeq 3' to *O. cumana*.

METABOLOMIC ANALYSIS OF THE RESISTANCE RESPONSE IN SUNFLOWER ROOTS TO THE PARASITIC WEED *OROBANCHE CUMANA*

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Orobanche cumana is a root parasitic angiosperm of sunflower that causes devastating losses in yield in Europe and Asia. This parasite is difficult to control and resistant cultivars play an important role in integrated control programmes. *O. cumana* seeds germinate in response to germination stimulants (e.g. dehydrocostus lactone) present in sunflower root exudates. The parasite radicle then differentiates (in response to a different suite of host derived signals) to form an attachment and penetration organ, known as the haustorium or tubercle. In a susceptible interaction, the parasite cells penetrate the host root cortex and endodermis and establish direct vascular connections to the phloem and the xylem of the host providing access to host nutrients. Resistance in sunflower cultivars can occur at different stages of parasite ingress into the root and microscopic studies have variously shown lignification of host cells, callose deposition, accumulation of phenolic compounds and cell death around the invading parasite. However, very little is known about the metabolic defence pathways underpinning different resistance reactions. Several studies have quantified specific defence metabolites but no global metabolomic analysis has been performed to identify the biochemical pathways up or down regulated during a resistant interaction. We performed a non-targeted metabolomic analysis of the roots of a susceptible and resistant sunflower cultivar following inoculation with *O. cumana* using Ultra-high Performance Liquid Chromatography -High Resolution Mass Spectrometry (UPLC-HRMS). We identified key defence pathways and metabolites involved in the resistance reaction. In addition we verified the identity and quantified key metabolites using standards and targeted UPLC-HRMS analysis of root samples. This study revealed that flavonoid and isoflavonoid biosynthetic pathways and the biosynthesis of coumarins, lignans and alkaloids (the latter derived from the shikimate pathway) were significantly upregulated during the defence response. For example, there was an accumulation of chlorogenic acid, ferulic acid, sinapic acid, caffeic acid glycoside and of the phytoalexin scopoletin. These data will be discussed in relation to our current knowledge of defence pathways in the sunflower-*O. cumana* interaction.

COMBINING ABILITY AND HETEROTIC PATTERNS OF QUALITY PROTEIN MAIZE INBREDS UNDER *STRIGA*-INFESTED ENVIRONMENTS

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Hybrid maize (*Zea mays* L.) production can revolutionize agriculture and contribute to alleviation of food insecurity and malnutrition in West and Central Africa (WCA). IITA has developed a large number of early QPM inbreds, there are no commercial early QPM hybrids in WCA. Information on the combining ability and heterotic patterns of maize inbreds is crucial for the success of a hybrid program targeting the stress environments of the sub-region. Ninety-one diallel crosses derived from 14 early maturing yellow-endosperm QPM maize inbreds were evaluated under *Striga*-infested environments at Mokwa and Abuja in Nigeria, 2011 and 2012. The objectives were to (i) examine the combining ability for grain yield of the set of early QPM yellow inbreds, (ii) determine the heterotic groups of the inbreds, (iii) identify the best testers, and (iv) determine the performance and stability of the inbreds in hybrid combinations under *Striga*-infested environments. Additive and nonadditive gene actions were important in the control of the inheritance of grain yield and other traits in the inbreds. General combining ability (GCA) effects for all traits were greater than specific combining ability (SCA) effects across *Striga*-infested environments suggesting that additive gene action was more important than the nonadditive in the set of inbred lines. The GCA mean squares for *Striga* damage at 8 and 10 weeks after planting (WAP) were significant and about three times greater than those of the SCA, indicating that additive gene action played a major role in the inheritance of the *Striga* damage. GCA and SCA mean squares were not significant for number of emerged *Striga* plants at 10 WAP but were significant at 8 WAP with preponderance of GCA over SCA, indicating that additive gene action modulates the inheritance of number of emerged *Striga* plants in the inbreds. The inbred lines were classified into three heterotic groups each based on the GCA effects of multiple traits (HGCAMT) and heterotic groups' specific and general combining ability (HSGCA) methods. There was close correspondence between the classification based on HSGCA and HGCAMT methods, indicating the effectiveness of the two methods in classifying inbreds. TZEQI 78, TZEQI 89, TZEQI 87, and TZEQI 82 were identified as the best inbred testers. Inbreds TZEQI 87 and TZEQI 91 had the highest GCA effects for grain yield while TZEQI 89 had the lowest. Grain yield ranged from 1008 kg ha^{-1} for TZEQI 80 × TZEQI to 5074 kg ha^{-1} for TZEQI 78 × TZEQI 92. The most outstanding hybrid, TZEQI 78 × TZEQI 92, out-yielded the best OPV check (2008 DTMA-Y STR) by 76%. The *Striga*-resistant hybrids were characterized by higher grain yield, better ear aspect, higher number of ears per plant, lower *Striga* damage, and lower number of emerged *Striga* plants at 8 and 10 WAP compared with the susceptible hybrids. The genotype main effect plus genotype × environment interaction (GGE) biplot analysis identified TZEQI 78 × TZEQI 92, TZEQI 79 × TZEQI 92, and TZEQI 78 × TZEQI 91 as the highest-yielding and stable hybrids across environments and should be promoted for adoption and commercialization in WCA.

EVALUATION OF RESISTANCE OF UPLAND RICE VARIETIES TO *STRIGA HERMONTHICA* THROUGH LABORATORY, POT AND FIELD EXPERIMENTS

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Striga hermonthica, a root parasitic weed, is a potential biotic constraint to upland rice production in Sub-Saharan Africa. *Striga* resistance of upland rice varieties was evaluated in laboratory, pot and field experiments. Among 52 varieties evaluated in the laboratory, SATREPS1, NERICA5 and NERICA13 exhibited high resistance. On their roots, only less than 3% of the inoculated *Striga* seeds developed into seedlings that were sustained. In contrast, more than 63% of *Striga* seedlings survived on the most susceptible variety, NERICA18. The three resistant varieties allowed 67–77% of *Striga* seedlings to penetrate into the roots, while most of the susceptible ones allowed less than 70%. Thus it would appear that the resistant varieties are endowed with post-penetration mechanisms that abort *Striga* parasitism. Moreover, two thirds of *Striga* that developed shoots on SATREPS1 died thus suggesting additional mechanism(s) that inhibit *Striga* growth after establishment of parasitism. Further evaluations of resistance of SATREPS1, NERICA5 and NERICA13 under pot and field conditions were conducted, in comparison with NERICA4 and NERICA18 as susceptible varieties and Nipponbare as a reported resistant variety. In the pot experiment, where *Striga* seedbank size was the same for all varieties, the number of emergent *Striga* plants per pot reached 0, 0.5, 2.5, 6.7, 13.0 and 4.7 for SATREPS1, NERICA5, NERICA13, NERICA4, NERICA18 and Nipponbare, respectively. Percent infested pots were 0, 33, 50, 100, 100 and 83, respectively, at the end of cultivation using six pots for each variety. In the field experiment, where *Striga* density was different by position, but the growth conditions for rice were relatively similar to farmer's fields, panicle dry weight (DW) per m^2 in *Striga*-infested field was 95, 110, 69, 31, 44 and 42% of that in *Striga*-free field, respectively. The number of emergent *Striga* plants per area and percent infested hills were much smaller in SATREPS1 and NERICA5 than in other varieties. Therefore, SATREPS1 and NERICA5 exhibited stable and reliable *Striga* resistance in all experiments. In addition, panicle DW in *Striga*-free field was more than 2-fold in SATREPS1 than in NERICA5. Accordingly, SATREPS1 was selected as a *Striga* resistant variety with adaptability to growth conditions in Sudan.

IDENTIFICATION OF NEW SOURCES OF RESISTANCE TO *STRIGA GESNERIOIDES* IN COWPEA ACCESSION

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The parasitic weeds, *Striga gesnerioides* and *Alectra vogelii* are major threats to cowpea production and productivity in the savannas of West and Central Africa. The level of infestation of *Striga* and *Alectra* are often so high that the crop can suffer 100% yield loss, and farmers are often forced to abandon their fields. Among the several control options proposed, breeding for resistance cultivar have been found to be most effective and economical for the resource poor farmers. Most of the cowpea cultivars with resistance to *Striga* biotypes prevalent in Nigeria were developed using B301 or lines derived from it as sources of resistance. Resistance in B301 is controlled by a single major gene (vertical resistance) that are of no durability. The breakdown of vertical resistance is common phenomenon in breeding for resistance. This could be attributed to new Striga races emerging or to an in increase in the aggressiveness of current Striga races. There have been reports of breakdown of Striga resistance in previously resistant cowpea cultivars in Nigeria. To delay such breakdown, pyramiding of more than one resistance gene from different sources into a single genotype would lead to the better ways of achieving durability of resistance. To facilitate accessibility to diverse new germplasm sources for breeding resistance to Striga/Alectra, 196 accessions collected from a mini core collection of the IITA cowpea germplasm were screened for resistance to *Striga*/*Alectra* under artificial infestation with Striga and Alectra in the screenhouse to identify new sources of resistance to the two parasites. Accessions were highly significant indicating accession difference in their reaction to Striga and Alectra resistance. Of the 196 accessions screened, three of the accessions were found to be completely and consistently free of *Striga* and *Alectra* attachment on the cowpea root. The three accessions were genotyped to validate phenotypic data and determine the relationships of the resistant accessions with the existing B301using two SCAR markers. Of the three accessions genotyped, two accessions produced polymorphic bands. Moderate level of genetic variation was found between existing B301 and one of the accessions. They amplified different band sizes indicating genetic variation or a new gene for *Striga* resistance. This accession found to show genetic variation from that of B301 is a potential donor parent for breeding and pyramiding of *Striga* resistance gene(s) into single genotype.

EVALUATION OF MARKER ASSISTED BREEDING *STRIGA* RESISTANT SORGHUM VARIETIES IN EASTERN AND CENTRAL AFRICA

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The parasitic weed, *Striga hermonthica* is a major constraint to sorghum production, particularly in semi-arid regions of Sub-Saharan Africa, where it can cause up to 100% yield loss in farmers' fields. Improving resistance to *Striga* by conventional phenotypic selection and breeding for resistance is hampered by the complexity of host parasite interactions and lack of reliable screening methods. In the recent past, the ability to develop markers and transfer target genomic regions has resulted in their extensive use in quantitative trait loci (QTL) mapping and marker assisted backcrossing (MABC). Previous research studies identified five QTL associated with stable *Striga* resistance from an Indian *Striga* tolerant durra landrace (N13). Through Marker Assisted Backcrossing, five QTLS were introgressed into five Farmer Preferred Sorghum Varieties (FPSVs) from Kenya, Sudan and Eritrea. Simple Sequence Repeats (SSR) markers for the QTL were identified and the genomic regions introgressed into the FPSVs through a series of backcrosses, with the resulting progenies being genotyped to detect the presence of QTL. A total of 51 *Striga* resistant lines were hence developed and evaluated for field resistance in Kenya, Sudan and Eritrea. Multi-locational trials are being conducted in Kenya and Eritrea as a prelude to official release of the varieties. To increase the efficiency of Marker Assisted Selection (MAS), 27 EST-SSR markers and DArTs in close association with *Striga* resistance QTLs were also identified and mapped using Sudan BC3S4. Following the mapping, populations of backcross (BC3S4) derived from N13 (*Striga* resistant) X three farmer preferred sorghum cultivars; Tabat, Wad Ahmed and AG-8 (*Striga* susceptible) were generated. Subsequently, thirtyone lines with confirmed *Striga* field resistance were developed and twenty lines with superior performance were selected for regional evaluation. Standard variety trials were carried in *Striga* sick plots over three seasons (2009 - 2011) in Gezira Research Station (GRS), Damazine, Sinnar, and Gedaref resulting in formal release of four varieties,T1BC3S4, AG6BC3S4, AG2BC3S4 and W2BC3S4. The thirtyone lines from Sudan, including the released varieties, are currently undergoing performance trials in Kenya, Uganda, Tanzania, Eritrea and Rwanda. The results have demonstrated the power of Marker Assisted Breeding in improving the efficiency and expediency in crop improvement.

Session 6: Environmental Factors, Modeling and Mapping

Chair

Hannan Eizenberg

TEMPO-SPATIAL MODELING OF BROOMRAPES (*OROBANCHE* AND *PHELIPANCHE* SPP.) PARASITISM - A KEY FOR THEIR SUSTAINABLE MANAGEMENT

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The broomrapes (*Orobanche* and *Phelipanche* spp.) are obligate root parasites. Most of their life cycle takes place in the soil subsurface, including seed germination, attachment to the host root, penetration and establishment in the host tissue. Toward the end of their life cycle they emerge from the soil and produce inflorescences bearing hundreds of thousands of seeds. In the underground stages of their life cycle, the root parasitic weeds are more sensitive to herbicides than in the aboveground developmental stages. Thus, knowledge of the parasitism stage is essential to effectively control the parasite using herbicides. Data on their infestation level or phenological stage are therefore essential for effective control. In this presentation two approaches for enhanced broomrape control efficacy will be discussed: a) quantification of the temporal variation and prediction of broomrape parasitism by a thermal time model; (b) estimation of the spatial variation of broomrape infestation within a field and between fields. During the last 12 years models for the parasitism dynamics has been developed for small broomrape (*O. minor*) in red clover, sunflower broomrape (*O. cumana*) in sunflower and Egyptian broomrape (*P. aegyptiaca*) in tomato, sunflower and carrot. The parasitism dynamics of all parasites-host systems is strongly temperature related and therefore enables us to use the thermal time (growing degree days, GDD) approach for predicting the parasitism dynamics. Since the four mentioned crops are grown during different seasons and therefore are exposed to wide-range of temperature regimes, the classical calculation of GDD cannot estimate accurately the parasitism dynamics. To overcome this inaccuracy, two GDD calculation methodologies, the linear and the beta function equations are presented. The variation of the spatial broomrape infestation was analyzed within a field and between fields by the use of Geographical Information Systems (GIS), allowing mapping of the spatial distribution of broomrape in the field and utilizing this data for preparing a Site Specific Weed Management (SSWM). Special attention is given to the development of an integrative management approach. An example of a decision support system 'PICKIT' for a rational management of Egyptian broomrape in processing tomato will be presented.

COMBINING RESISTANCE TO *STIGA HERMONTHICA* WITH TOLERANCE TO DROUGHT IN MAIZE

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Maize is grown in fields infested with *Striga hermonthica* in the major production zones of West and Central Africa, expositing the crop to the destructive root parasite. The effect of *S. hermonthica* is severe on crops wreaked by drought, which frequently occurs in the major maize production zones in this region. The main challenge for breeders is to develop new maize cultivars that can produce high grain yields in areas infested by the parasite that are also affected by drought. As climate changes, with more frequent extremes in weather conditions, it becomes ever more imperative to develop new maize varieties that combine resistance to *S. hermonthica* with tolerance to drought in order to sustain main production. Breeding at the International Institute of Tropical Agriculture has focused on the development of maize germplasm to help maximize yield under drought stress and minimize losses under severe *S. hermonthica* infestation. Initially, *S. hermonthica* resistant maize inbred lines were screened under controlled drought stress imposed by withdrawing water from four weeks after planting to harvest. *S. hermonthica* resistant inbred lines with some levels of drought tolerance were selected and used for developing single- three-way-, and top-cross hybrids, which were included in separate trials, which were evaluated under controlled drought stress, optimum growing conditions, and artificial *S. hermonthica* infestation in Nigeria since 2008. Significant differences in grain yield were detected among hybrids in the various trials planted under the three growing conditions notwithstanding the presence of significant hybrid x location interactions. The best hybrids selected from the different trials out-yielded the commercial hybrid check by more than 50% under controlled drought stress and by more than 80% under artificial *S. hermonthica* infestation. They also sustained less *Striga* damage symptoms and supported fewer emerged parasites in comparison with commercials hybrid check. Most of the best hybrids were found to be competitive to the commercial hybrid checks under optimum growing conditions. The ability of the best hybrids to produce high grain yields in the different production environments represents the potential that exists to enhance phenotypic plasticity in a single hybrid to adapt to changes in growing conditions. Several bi-parental crosses between *S. hermonthica* resistant and drought tolerant maize inbred lines have also been made to further enhance the levels of resistance to the parasite and tolerance to drought in single hybrids targeted to areas affected by the two stresses.

DEVELOPMENT OF A DECISION SUPPORT SYSTEM BASED ON MODELING APPROACH FOR EGYPTIAN BROOMRAPE (*PHELIPANCHE AEGYPTIACA*) CONTROL IN CARROT

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The root parasite *Phelipanche aegyptiaca* is a major pest in carrots in Israel. Growing carrot in infested fields may result in severe damages and even total yield loss. For effective *P. aegyptiaca* control, farmers must know when to apply the herbicide, which herbicide to apply and when to repeat herbicide applications for a continuous parasite control. Thirty five field and controlled environment studies were conducted to address these questions. As a result, a decision support system was developed based on modeling the parasitism dynamics and optimizing herbicide type and rate. A parasitism dynamics sub-model was composed by a mathematical beta function model to compute the growing degree-days (GDD) from soil temperature, and four parameters Weibull model were used to describe the parasitism dynamics of *P. aegyptiaca* in carrots. According to the model it was found that first attachment appears at 500 GDD, 63% of total attachments appear at 600 GDD and the maximum number of attachment appear at 850 GDD. In a parallel study, the selectivity of several herbicides to carrot was examined at different temperature regimes and carrots phenological stages, in order to select an effective herbicide for *P. aegyptiaca* control. Out of imazamox, imazapic, sulfosulfuron, trifloxysulfuron and glyphosate, the last herbicide was found to be effective for *P. aegyptiaca* control and safe to the carrots. Temperature and carrots phenological stage significantly affected the selectivity to carrot. At high temperatures and early growing stage the carrot is less tolerant to the herbicide. It was found that glyphosate at rate of 54 g ae ha^{-1} effectively controlled broomrape and is safe to carrot. Combining the two sub models for a decision support system proposes that for effective broomrape control in carrot three sequential glyphosate treatments (54 g ae ha^{-1} each) should be applied at 600 GDD, 850 GDD and 1100 GDD (GDD computed using beta function) from carrots planting. Four field studies that were performed during 2012-2013 validated the model resulting in an effective *P. aegyptiaca* control without crop damage.

USING MICROTOMOGRAPHY TECHNIQUES TO BETTER UNDERSTAND THE ANATOMICAL INTERFACE BETWEEN HOST AND PARASITE

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In many occasions, when studying the interface between host and parasite, researchers may face some difficulties to clearly understand spatially the anatomical structures observed. Details about *haustoria* may be assessed by plant anatomy technics such as microtomy, bright field microscopy, fluorescence microscopy and transmission electron microscopy. However, all these technics offer bidimentional images hard to be interpreted in three dimentions. Counting on the recent advances on microscopy and informatics, such as confocal laser scanning microscope, low vacuum scanning electron microscopy and tri-dimension reconstitution performed by sophisticated computer software, the knowledge on host-parasite interface has been improved. Although, many physical limitations still remain, restricting the use of some methods. Also, the preparations needed for all the mentioned technics are highly time-consuming and sometimes depend on subtle manual skills. In addition, the structure of the endophytic system of some parasitic species may also be difficult to assess with such methods. In this context, the use of microtomography technics (MCT) appears as a helpful way to better understand structures and connections formed by parasitic plants. This method allows us to study the host-parasite connection as a whole, observing it in a tridimensional way. It also brings the possibility of observing inner structures with a very fine resolution, even when dealing with very thick woody galls. There is a variety of MCT devices available and they vary in penetration, resolution and speed of image reconstruction. We used a microtomograph for small samples (Bruker, SkyScan1176), testing a variety of parameters. We also tested for the best material conditions, using materials that were fresh, dried or included in media for histology. We studied *haustoria* and galls of different parasites (e.g, Apodanthaceae: *Pilostyles ulei*; Loranthaceae: *Psittacanthus robustus*, *Struthanthus* spp*;* Viscaceae: *Phoradendron* spp; Balanophoraceae: *Scybalium fungiforme*). Our results showed a wide degree of variation in the quality of images, depending upon the water content and the presence of including media in the materials analyzed. In general, images of woody galls included in histology media did not offer good results once the media absorbed a great amount of the X-ray radiation. One of the most interesting achievements was the possibility of the observation of endophytes with all its length inside host branches. This allowed us to distinguish whether two infestation sprouts were connected, consisting of a single individual. Another interesting result was the possibility of seeing the body of endoparasites at developmental stages before that of arising from host barks. Thus, it became possible to drive better afterwards histological preparation. At the present phase our research group is developing and testing the use of several contrast substances in order to improve the understanding of particular xylem structures, especially those at the interface between two connecting hydraulic systems such as the host-parasite interface.

FACTORS AFFECTING PARASITIC WEED INFESTATION IN RAIN-FED LOWLAND RICE SYSTEMS: THE CASE OF *RHAMPHICARPA FISTULOSA* IN BENIN

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Parasitic weeds pose increasing problems to rice production in sub Saharan Africa. The parasitic weed, *Rhamphicarpa fistulosa*, is particularly threatening rain-fed lowland rice production in Benin. Data from 233 rice fields located in 12 *Rhamphicarpa*-infested inland valleys in Central and North Benin were collected to assess the factors affecting infestation by this parasitic weed, and the ability of farmers to cope with this problem. Data were analysed using the double-hurdle modelling approach. Results showed that 74% of the surveyed fields were infested by *Rhamphicarpa* with an average infestation severity of 82 plants/m^2. The likelihood of infestation of rice fields is higher on poorly fertile soils and in inland valleys located in the cotton zone of central Benin (Sudan-Guinea savannah). The use of herbicides at the recommended time and ploughing reduce the likelihood of infestation. Management practices such as late application of herbicide, hoe or hand weeding and medium-rate fertilizer application reduce the severity of infestation. Infestation risk and severity of actual infestation levels are negatively correlated. This suggests that when farmers are aware of the risk of infestation, they take management measures resulting in reduced infestation levels. Farmers' awareness of the problem should therefore be increased. We conclude that the physical character of the rice growing environment (agro ecological zone, location in the IV bed, soil fertility status), the use of inputs, and land preparation determine the risk of infestation while farmers' ability to cope with the infestation is mainly determined by socio-economic characteristics, and farming practices such as late sowing, fallow length, and number of weeding efforts. These results suggest that farmers can reduce both the likelihood of infestation of their crop and the related severity of infestation.

Session 7: Host-Parasite Communnication

Chairs

Koichi Yoneyama and Maurizio Vurro

REGULATION OF PARASITIC PLANT GERMINATION

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The lifecycle of root parasitic plant species is closely regulated by the presence of their hosts and signaling molecules released by the host play an important role in this interaction. This begins with the first step in the parasites' lifecycle, germination, which is tightly regulated by host-produced stimulants that induce the germination of the parasite seeds. Several classes of germination stimulants have been identified. One of these, the strigolactones, have recently also been identified as a new group of plant hormones, that plays an important role in the regulation of shoot branching/tillering and root architecture in plants. In addition SLs are chemical signals stimulating plant root colonization by symbiotic arbuscular mycorrhizal (AM) fungi. The biosynthetic pathway of SLs has been partially elucidated, using highly branched/tillered mutants and it was shown that the carotenoid isomerase D27, the carotenoid cleavage dioxygenases CCD7 and CCD8 and a cytochrome P450, MAX1, are involved in strigolactone biosynthesis. Recently also a transporter was identified in Petunia, PhPDR1, that seems to be responsible for the secretion of strigolactones into the rhizosphere. The multiple functions of the strigolactones in the plant and in the rhizosphere seem to converge on the regulation of many of these functions by phosphate starvation. The implications of the multiple functions of strigolactones and their regulation for the infection by and control of parasitic plants will be discussed.

DIFFERENCE IN *STRIGA*-SUSCEPTIBILITY CORRELATES WITH 5-DEOXYSTRIGOL EXUDATION BUT NOT WITH COMPATIBILITY/SELECTIVITY TO AM FUNGI IN MAIZE

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Striga spp., are devastating root parasitic weeds that attack monocot crops including sorghum, millet and maize in semi-arid tropics. Their seeds require germination stimulants (mainly strigolactones, SLs) released from host roots to germinate. Our previous study demonstrated that a qualitative difference in SL exudation exists between the maize cultivars Pioneer 3253 and KST 94, susceptible and tolerant to *Striga*, respectively. 5-deoxystrigol was detected in root exudates from Pioneer 3253 but not in those from KST 94. Since SLs also work as host recognition signals for arbuscular mycorrhizal (AM) fungi, we examined if qualitative and quantitative differences in AM colonization exist between these cultivars. Mycorrhizal colonization was only slightly higher in Pioneer 3253. No significant differences of AM fungal community compositions were observed between the cultivars.

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

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Parasitic weeds, such as broomrapes (*Orobanche* and *Phelipanche* spp) and witchweeds (*Striga* spp), can cause severe damage on crop plants throughout the world and few efficient methods are available to control these pests. Parasitic weeds extract water, minerals and photoassimilate resources from their hosts. *Orobanche cumana* attacks exclusively sunflower and represents one of the major problems on this crop. Germination is an important step in the developmental cycle of parasitic weeds. Their seeds use signal molecules released by the host to recognize the presence of a root. Different molecules were identified as germination stimulants including strigolactones and dehydrocostuslactone (DCL).

One way to control parasitic weed germination could be the use of Arbuscular Mycorrhizal (AM) fungi. AM symbiosis is a mutualistic interaction between soil fungi and the roots of most terrestrial plants. Previous studies have shown that mycorrhization can reduce the infection of sorghum by *Striga hermonthica*. The aim of our work was to determine whether this also applies to the sunflower/*O. cumana* interaction. We first observed that sunflower mycorrhization reduced significantly the infection of sunflower by *O. cumana*. We then showed that mycorrhizal sunflower root exudates induced lower *O. cumana* seed germination than non-mycorrhizal root exudates. Germination stimulants were analyzed, and mycorrhizal root exudates were found to contain less strigolactones and as much DCL as non-mycorrhizal root exudates. Moreover, mycorrhizal root exudates were able to inhibit *O. cumana* seed germination induced by a synthetic strigolactone. We then showed that AM fungi alone could produce inhibitors of *O. cumana* germination, and that this inhibitory effect seemed restricted to broomrape seeds. This novel property of AM fungi could be used as an alternative biocontrol strategy against broomrapes.

THE HAUSTORIUM OF THE OROBANCHACEAE – A REVIEW

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The haustorium forms the structural and physiological bridge between the parasitic plant and its host. It facilitates the movement of water, nutrients and various macro-molecules from the host to the parasite while addressing the differences between the two organisms. The structure of mature haustoria is highly diverse in the Orobanchaceae. Two types of haustorium can be found: the terminal ('primary') haustorium that develops at the apex of the radicle of obligate parasites soon after germination, and the lateral ('secondary') haustorium, which arises from parasite roots. In some species the haustorial structure is relatively simple, while in other species it is highly complex. Nonetheless, all mature haustoria are composed of three distinct structural regions: (a) the Haustorial Base, which connects the haustorium with parasite tissues; (b) the Endophyte, which lies within the host and is continuous with at least some host tissues, and (c) the Haustorial Bridge, which lies between them. All functional haustoria include xylem that directly connects with both host and parasite xylem, thus forming a continuous apoplast between the two organisms. Direct phloem connections were found only between a few obligate parasitic Orobanchaceae and their hosts. Both conductive tissues are associated in the haustorium with specialized parenchyma cells. Some structures, like the hyaline tissue and the graniferous tracheary elements, are unique to the haustorium and seem to have special roles not only in nutrient transport, metabolism and storage, but also in other functions that allow the parasite to be directly connected to another plant. These include the regulation of hydrostatic pressures, prevention of cavitation and recovery from embolism, and protection against host pathogens. This review presents the major structural aspects that characterize the haustorium in the Orobanchaceae and discusses their possible function.

COMPARATIVE HAUSTORIAL MORPHOLOGY AND STRUCTURE IN PARASITIC OROBANCHACEAE

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Orobanchaceae present an ideal system for studying the evolution of the parasitic habit in flowering plants: with a single origin of parasitism, the full range of trophic modes is represented, from non-parasites to facultative and obligate hemiparasites and holoparasites. Most members of Orobanchaceae are parasitic on the roots of their host plants, and haustoria are present in terminal and lateral positions along the parasite's root system. Haustoria have a generalized internal structure, being composed of a vascular core, hyaline body, and endophyte, often with a bridge of xylem cells connecting the vasculature of host and parasite. Reports of phloem connections between host and parasite in Orobanchaceae are rare. Since the recircumscription of the family to include all parasitic members of Lamiales, detailed comparative studies of haustorial structure within and across the main lineages of the family are lacking. Haustoria were collected from plants excavated in the field, representing four of five parasitic lineages within the family, and were fixed in 2.5% glutaraldehyde, and subsequently embedded in paraffin and plastic (JB-4) for structural examination. Direct xylem connections have been observed in all taxa examined; the width of the xylem bridge in haustoria is variable among the parasitic lineages in the family. The hyaline body, whose function remains unknown, was not obviously present in all taxa examined. The proximity of parasite and host phloem, and the presence of interfacial parenchyma at the host : parasite interface were variable, and characteristics of these elements will be further discussed. Is phloem essential to sustaining the host : parasite relationship?

DYNAMIC CHANGES IN CELL MORPHOLOGY DURING HAUSTORIUM DEVELOPMENT IN *PHTHEIROSPERMUM JAPONICUM*

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Parasitic plant species in Orobanchaceae, such as *Striga* and *Orobanche*, are serious agricultural threats in worldwide. All of the parasitic plants in this family form a haustorium, the special organ, which penetrates a host root and makes xylem connections. Through this connection, parasitic plants obtain water and nutrient from host plants. Therefore haustorium is the most important organ for the host-parasite interaction; however haustorium development processes are poorly understood at cellular level. To study the mechanisms of haustorium development we use *Phtheirospermum japonicum*, a facultative parasite belonging to Orobanchaceae, as a model. *P. japonicum* is likely to share the parasitic mechanisms with other pathogenic Orobanchaceae plants.

We analyzed haustorium development by a combination of technovit sectioning and time-lapse microscopic observation. The haustoria formed on host and non-host roots were embedded in Technovit resin and thin sectioned. Haustorial cell morphology was carefully observed, under bright-field microscopy. We found that mature haustorial cells can be classified into 4 types according to their size and position; 1. narrow elongated cells at the interface between host and parasite cells, designated palisade cells, 2. small cells positioned in line along the host-parasite xylem connection, 3. large cells which occupy the peripherical parts of haustorium and 4. xylem cells distinguished by secondary cell wall. To analyze how these cell types are generated, cell division during haustorium development was observed in transgenic *P. japonicum* roots carrying *AtCYCB1;2-YFP* construct, which specifically expresses in dividing cells. In early haustorium development stages, cell division occurs in a broad area around the haustorium emerging site, and the dividing area is subsequently centralized. Later, cell division occurs in line connecting parasite and host xylems. This study sketches dynamics of cell differentiation during haustorium development and explores developmental aspects in plant parasitism.

FUNCTIONAL IDENTIFICATION OF THE GENES INVOLVED IN HAUSTORIUM DEVELOPMENT IN THE FACULTATIVE PARASITIC PLANT *PHTHEIROSPERMUM JAPONICUM*

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Parasitic plants in Orobanchaceae cause devastating damages in agriculture worldwide. A common feature of these parasites is the presence of haustorium, a root feeding structure responsible for connecting the parasite to the host. The facultative parasitic plant *Phtheirospermum japonicum,* belonging to Orobanchaceae, is applicable for various genetic and reverse-genetic analyses, such as crossing and transformation; therefore *P. japonicum* represents a suitable model for parasitism studies. We used the large-scale transcriptome of *P. japonicum* to identify the genes involved in haustorium development. mRNA sequences on parasitic or non-parasitic stages were sequenced and *de novo* assembled. A custom microarray was designed based on the assembly and the gene expression was analyzed in 8 different time points upon the treatment with DMBQ (2,6-dimethoxy-*p-benzoquinone*), a natural compound which induces haustorium development *in vitro*. Comparison of gene expression profiles between DMBQ-treated and non-treated roots identified 1577 differentially expressed genes divided into three clusters according to Self-Organizing Maps algorithm. After validation of differential gene expression by qRT-PCR, we selected 6 genes for functional analysis. Significant decrease in haustorium number was observed in the roots silenced for genes encoding *YUCCA* flavin monooxygenases (PjYUC-24658), a key enzyme in auxin biosynthesis, compared to the control roots transformed with empty vector. PjYUC-24658 protein shares 63-66% amino acid sequence identity with *Arabidopsis YUCCA* genes. The expression pattern analysis of other *P. japonicum YUCCA* genes indicated that PjYUC-24658 is exclusively upregulated during the DMBQ treatment. Our results indicate the importance of *YUCCA* gene for haustorium development and further investigation will provide new insights into the role of auxin during haustorium development.

STRUCTURAL REQUIREMENTS OF STRIGOLACTONES FOR GERMINATION INDUCTION AND INHIBITION OF *STRIGA GESNERIOIDES* SEEDS

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Thirty-six stereoisomers of the naturally occurring strigolactones, strigol, sorgolactone, orobanchol, sorgomol and 5-deoxystrigol were prepared and screened for the ability to induce and/or inhibit the germination of *Striga hermonthica* and *S. gesnerioides* seeds collected from mature plants that parasitized sorghum and cowpea, respectively. All of the compounds induced *S. hermonthica* seed germination, albeit displayed differential activities. On the other hand, only a limited number of the compounds induced significant germination in *S. gesnerioides*, thus indicating strict structural requirements. Strigolactones inducing high germination in *S. gesnerioides* induced low germination in *S. hermonthica*. Strigolactones with the same configuration at C3a, C8b and C2' as that in 5-deoxystrigol induced high germination of *S. hermonthica* seeds, but most of them inhibited the germination of *S. gesnerioides*. From an ecological point of view, it is interesting that *S. gesnerioides* seed germination is induced by the strigolactones alectrol and *ent*-2'-*epi*-orobanchol, produced by cowpea, while it is strongly inhibited by sorgolactone, sorgomol and 5-deoxystrigol, which are exuded from the roots of sorghum, the most preferable host for *S. hermonthica*. Sorghum and cowpea are grown in the same ecological zone in West and Central Africa, where both *S. gesnerioides* and *S. hermonthica* are predominant. The differential responsiveness of the 2 parasitic weeds to strigolactones could be an adaptation of *S. gesnerioides* to avoid being triggered to germinate by non-host plants. Germination inhibition by natural strigolactones, reported for the first time, may provide a new approach for biological control of *S. gesnerioides*.

NOVEL GERMINATION STIMULANTS FOR ROOT PARASTIC PALNTS PRODUCED BY *NICOTIANA TABACUM* L.

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Broomrapes (*Orobanche* and *Phelipanche* spp.) and witchweeds (*Striga* spp.) are root parasitic weeds causing enormous losses of agricultural production. The seeds of these parasites germinate when they perceive germination stimulants released from their host and some nonhost plants. Among the germination stimulants, strigolactones (SLs) appear to be of primary importance. Moreover, they induce hyphal branching of symbiotic arbuscular mycorrhizal fungi in the rhizosphere and function as phytohormones regulating plant shoot and root architectures. Tobacco (*Nicotiana tabacum* L.) is a host of *Phelipanche ramosa* L., which causes severe damage and yield loss (up to 70%) in tobacco. Recently we found that the root exudates of tobacco contained at least 13 different SLs including solanacol, solanacyl acetate, orobanchol, orobanchyl acetate, *ent*-2'-*epi*-orobanchol, *ent*-2'-*epi*-orobanchyl acetate, 5-deoxystrigol, *ent*-2'-*epi*-5-deoxystrigol, and 5 novel SLs whose structures have not yet been identified.

In this study, isolation and structure determination of the novel SLs produced by tobacco were conducted. About 500 tobacco plants (cv. Tsukuba No.1) were grown hydroponically and the root exudates collected. The root exudates were subjected to solvent partitioning to give a neutral ethyl acetate (EtOAc) soluble fraction. This was bioassay-guided fractioned to obtain active germination stimulants including novel SLs. LC-MS/MS and GC-MS analyses indicated that three novel SLs are putative didehydro-orobanchol isomers. The structure elucidation of the novel SLs will be presented.

IDENTIFICATION OF STRIGOLACTONES PRODUCED BY *HOUTTUYNIA CORDATA*, A CHINESE MEDICINAL PLANT

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Strigolactones (SLs) are plant secondary metabolites which induce seed germination of parasitic plants, witchweeds (*Striga* spp.) and broomrapes (*Orobanche* and *Phelipanche* spp.), and hyphal branching of arbuscular mycorrhizal (AM) fungi in the rhizosphere. In addition, SLs function as a novel class of plant hormones regulating shoot and root architecture.

Natural SLs, which have been characterized from root exudates of various plants, have a tricyclic lactone (ABC part) connecting to a butenolide group (D-ring) *via* an enol ether bridge. The natural SLs can be divided into two groups, strigol-type and orobanchol-type, according to their configurations of the C ring.

In this study, SLs produced by *Houttuynia cordata*, a Chinese medicinal plant, were isolated and characterized. *H. cordata* was found to produce four strigol-type SLs including a novel SL, named strigone. In addition to these SLs, *H. cordata* produces strigol isomers. Isolation and structural elucidation of strigol isomers are in progress. In stereochemistry-activity relationship studies, strigone stereoisomers showed significantly different germination stimulation activities on the seeds of three root parasites, i.e.: *Orobanche minor*, *Phelipanche ramosa*, and *Striga hermonthica*.

NOVEL STRIGOLACTONES PRODUCED BY BLACK OAT

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Among the plant-derived germination stimulants for root parasitic plants, strigolactones (SLs) are the most widely distributed in the plant kingdom. SLs are branching factors of symbiotic arbuscular mycorrhizal fungi, and a novel class of plant hormones inhibiting shoot branching. All the plants investigated so far have been found to produce SLs and currently the total number of the identified natural SLs have exceeded 30. In this study, the characterization of germination stimulants produced by black oat (*Avena strigosa*), an allelopathic plant, was conducted. Although root exudates of black oat induced high germination of *Orobanche minor* seeds, none of known SLs could be detected. Distribution of germination stimulation activity after RP-HPLC revealed that black oat produced at least 6 novel germination stimulants presumably including novel strigolactones. Bioassay-guided purification of crude extract afforded several active fractions. Two of them were purified and purified samples were subjected to NMR measurement. The structures of these stimulants, estimated from their NMR data, are unique and significantly different from known SLs. Details of the chemical structures of germination stimulants produced by black oat will be presented.

EFFECTS OF EXOGENOUS SUBSTANCES ON PARASITISM OF *CISTANCHE DESERTICOLA*

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Exogenously applied norflurazon, a herbicide inhibiting carotenoid biosynthesis (inhibitor of phytoene desaturase), and 2,6-dimethoxybenzoquinone (2,6-DMBQ) are known to promote seed germination and haustorium formation, respectively, in root parasitic plants. In the present study, the effects of these two compounds on seed germination, haustorium formation, endogenous hormone (ABA and IAA) levels, and host specificity of a root parasitic plant *Cistanche deserticola,* an important medicinal plant in China, were studied to develop effective propagation method of this plant. The results showed that norflurazon could promote seed germination, reduce ABA levels and increase IAA levels in the seeds, and break host specificity of *C. deserticola*. 2,6-DMBQ could promote haustorium formation, increase IAA levels in seedlings and strengthen the promotive effect of norflurazon on parasitism of *C. deserticola*. The results indicated that the substances released from roots of its host *Haloxylon ammodendron* could promote seed germination and haustorium formation by regulating endogenous hormone levels in *C. deserticola.*

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