



15th World Congress on Parasitic Plants

June 30 – July 5 2019,
Amsterdam, The Netherlands



Sponsors 15th World Congress on Parasitic Plants,
30 June – 5 July 2019, Amsterdam, the
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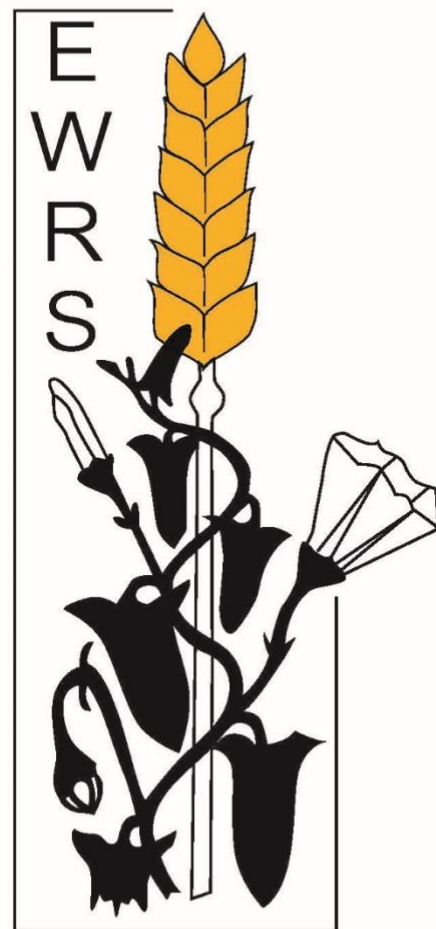


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SCHEDULE OF ORAL PRESENTATIONS

SUNDAY, JUNE 30

- 16.00 - 20.00 Registration open
18.00 - 21.00 Welcome mixer with snacks sponsored by Trends in Plant Science | Cell Press

MONDAY, JULY 1

- 09.00 - 09.20 Welcome by Julie Scholes and Harro Bouwmeester
- 09.20 – 12.40 Host plant resistance**
Session chair: *Steve Runo*
- 09.20 – 09.55 The parasite *Cuscuta australis* with a streamlined genome mediates inter-plant systemic signals
Jianqiang Wu
- 09.55 – 10.30 *Cuscuta* microRNAs target host mRNAs involved in defence and vascular function
Michael Axtell
- 10.30 – 11.00 Coffee break
- 11.00 – 11.25 Interspecific long-distance movement of *Cuscuta* small RNAs control biological processes in host-parasitic plant complex
Koh Aoki
- 11.25 – 11.50 A peptide motif of a parasitic plant cell wall protein is recognized by the receptor protein CuRe1 and induces defence in tomato
Markus Albert
- 11.50 – 12.15 Molecular basis for tomato resistance to the parasitic plant *Cuscuta*
Neelima Sinha
- 12.15 – 12.40 Characterization of resistance to sunflower broomrape (*Orobanche cumana* W.) in sunflower (*Helianthus annuus* L.)
Dana Sisou
- 12.40 – 12.50 International consortium on sunflower broomrape resistance
Stephane Munos & Begoña Pérez-Vich
- 12.50 – 14.15 Lunch
- 14.15 – 18.00 Ecology, phylogeny and evolution**
Session chair: *Claude DePamphilis*
- 14.15 – 14.50 Eco-evolutionary causes and consequences of parasitism in plants
Susan Wicke
- 14.50 – 15.25 Novel genetic code and record-setting AT-richness in the highly reduced plastid genome of the holoparasitic plant *Balanophora*
Claude dePamphilis
- 15.25 - 15.50 A neglected alliance in battles against parasitic plants: AM and rhizobial symbioses alleviate damage to a legume host by root hemiparasitic *Pedicularis* species
Airong Li
- 15.50 – 16.20 Coffee break
- 16.20 – 16.45 When the same is not the same
Peter Toth
- 16.45 – 17.10 Genetic diversity of *Orobanche cumana* (sunflower broomrape) populations at the world level revealed by SSR markers
Luyang Hu
- 17.10 - 17.35 Facultative parasitism: an evolutionary precursor of complete parasitism or an effective strategy in its own right?
Lammert Bastiaans
- 17.35 – 18.00 Fitness of reciprocal F1 hybrids between *Rhinanthus minor* and *R. major*
Renate Wesselingh
- 18.00 - 19.30 Poster viewing with drinks
- 19.30 - 21.00 Dinner
- 21.00 – Bar conference centre open

TUESDAY, JULY 2

- 09.00 – 12.45 Genes and genomes**
Session chair: *Jim Westwood*
- 09.00 – 09.35 *Cuscuta campestris*: A plant genome under the influence of a parasitic lifestyle
Kirsten Krause
- 09.35 – 10.10 Genetic basis for host and parasitic plant communication



Satoko Yoshida

10.10 – 10.35	Exploring the evolutionary origin of haustorium development in root parasitic plants <i>Daniel Steele</i>
10.35 – 11.05	Coffee break
11.05 – 11.30	Where the action is: gene expression at the parasite-host interface <i>Elizabeth Kelly</i>
11.30 – 11.55	Convergent horizontal gene transfer and crosstalk of mobile nucleic acids in parasitic plants <i>Claude dePamphilis</i>
11.55 – 12.20	The identification of candidate pathogenicity-related genes from the genome of <i>Striga hermonthica</i> <i>James Bradley</i>
12.20 – 12.45	Lack of evidence for horizontally transferred genes in mitochondria of <i>Cuscuta</i> species <i>Benjamin Anderson</i>
12.45 – 14.15	Lunch
14.15 – 18.10	Molecules and Biochemistry Session chair: David Nelson
14.15 – 14.50	CHEMICALS that control <i>Striga</i> germination <i>Tadao Asami</i>
14.50 – 15.25	Signalling pathways in <i>Striga hermonthica</i> germination <i>Shelley Lumba</i>
15.25 – 15.50	Complementary hormone-based approaches for <i>Striga</i> Control <i>Salim Al-Babili</i>
15.50 – 16.20	coffee break
16.20 – 16.55	Unravel strigolactone signaling and controlling parasitic plant behaviors in <i>Striga</i> <i>Yuichiro Tsuchiya</i>
16.55 – 17.20	Structural and biochemical characterization of strigolactone parasitic receptors, understanding their functionality and how to inhibit them <i>Amir Arellano Saab</i>
17.20 – 17.45	Identification and characterization of α -galactosidase capable of hydrolyzing planteose in <i>Orobancha minor</i> as a target for control of root parasitic weeds <i>Atsushi Okazawa</i>
17.45 – 18.10	Cannalactone: a new non-canonical strigolactone exuded by <i>Cannabis sativa</i> roots with a pivotal role in host specialization within French broomrape (<i>Phelipanche ramosa</i>) populations Jean-Bernard Pouvreau
18.10 – 19.30	Poster viewing with drinks
19.30 – 21.00	Dinner
21.00 –	Bar conference centre open
09.00 – 12.45	Control and Management Session chair: Maurizio Vurro
WEDNESDAY, JULY 3	
09.00 – 09.35	<i>Striga</i> research on finger millet: protocols, GWAS and RNA sequencing <i>Damaris Odeny</i>
09.35 – 10.10	Parasitic weed management - opportunities and challenges <i>Hanan Eizenberg</i>
10.10 – 10.35	Advances in parasitic weed control in the field <i>Binne Zwanenburg</i>
10.35 – 11.05	Coffee break
11.05 – 11.30	Correctly multi-targeted Host-Induced Gene Silencing (HIGS) should allow full and sustainable control of parasitic weeds <i>Jonathan Gressel</i>
11.30 – 11.55	Realizing the suicidal germination strategy to control <i>Striga hermonthica</i> in rain-fed agriculture of sub-Saharan Africa <i>Boubacar Kountche</i>
11.55 – 12.20	How do fertilisers affect the facultative parasitic weed <i>Rhamphicarpa fistulosa</i> ? <i>Jonne Rodenburg</i>
12.20 – 12.45	Development of chickpea (<i>Cicer arietinum</i> L.) mutant resistant to imidazolinone herbicides for broomrape management <i>Evgenia Dor</i>
12.45 – 13.45	Lunch
14.00 – 23.00	Conference tour + Conference dinner



THURSDAY JULY 4

09.00 – 12.45	Parasitic plant biology
	Session chair: <i>Airong Li</i>
09.00 – 09.35	Understanding the arms race: host resistance and parasite virulence in the <i>Striga</i> -cereal interaction <i>Julie Scholes</i>
09.35 – 10.10	Native parasitic plants: a solution of plant invasions worldwide? <i>Jakub Tesitel</i>
10.10 – 10.35	Transcriptomics to farmer field: a system biology approach for commercializing root parasitic <i>Santalum album</i> (Sandalwood) <i>Pradeepa Bandaranayake</i>
10.35 – 11.05	Coffee break
11.05 – 11.30	Reproductive biology and pollination of <i>Cynomorium songaricum</i> (Cynomoriaceae) <i>Guilin Chen</i>
11.30 – 11.55	<i>Triphysaria</i> controls vegetative self-recognition by restricting release of HIFs in roots <i>Yaxin Wang</i>
11.55 – 12.20	Analysis of genetic variation in pre and post attachment resistance mechanisms in maize inbred lines to the parasitic weed <i>Striga hermonthica</i> ; implications for control <i>Mamadou Cissoko</i>
12.20 – 12.45	Impact of the soil microbiome on <i>Striga</i> -sorghum interaction <i>Desalegn Etalo</i>
12.45 - 14.15	Lunch
14.15 – 18.00	Parasitic plant-host interaction
	Session chair: <i>John Yoder</i>
14.15 – 14.50	War and peace – the molecular dynamics of compatible and incompatible <i>Striga</i> -host plant associations <i>Mike Timko</i>
14.50 – 15.25	What model plants can tell us about parasitic plants <i>Thomas Spallek</i>
15.25 - 15.50	Message received: Evidence for translation of mobile mRNAs in <i>Cuscuta</i> -host interactions <i>James Westwood</i>
15.50 – 16.20	Coffee break
16.20 – 16.45	The roles and functions of lignin in parasitic plant-host interaction <i>Songkui Cui</i>
16.45 – 17.10	A receptor and pathways discovered in the lignin-based resistance to <i>Cuscuta campestris</i> in Heinz hybrid tomato cultivars <i>Min-Yao Jhu</i>
17.10 - 17.35	Cytokinins act as signaling molecules within the rhizosphere to trigger haustorium formation in the holoparasitic plant <i>Phelipanche ramosa</i> <i>Estelle Billard</i>
17.35 – 18.00	Striking vegetative developmental convergence in endoparasitic angiosperms <i>Luiza Teixeira-Costa</i>
18.00 - 19.30	Poster viewing with drinks
19.30 - 21.00	Dinner
21.00 –	Bar conference centre open
	Departure participants





Monday 1 July 9.00 – 12.40 Host plant resistance

The parasite *Cuscuta australis* with a streamlined genome mediates inter-plant systemic signals

Jianqiang Wu, Christian Hettenhausen, Yuxing Xu, Jingxiong Zhang, and Nian Liu

Kunming Institute of Botany, Chinese Academy of Sciences, Lanhei Road 132, Kunming 650201, Yunnan Province, China

Cuscuta spp. (dodders) are stem parasites that naturally graft to their host plants to draw water and nutrients, and multiple adjacent hosts can often be parasitized by a dodder plant simultaneously, forming dodder-connected plant clusters. Whether ecologically meaningful signals can be transferred between dodder and hosts and among hosts in the dodder-connected plant clusters remains unclear. We show that when two host plants are bridge-connected by *Cuscuta* parasites, insect herbivory on one host induced strong defense-related responses in the dodder-connected other hosts, and these hosts exhibited increased resistance to insects (Hettenhausen et al., 2017). Furthermore, using ¹⁵N labeling experiments, we found that dodder can transfer N from a N-replete host to dodder-connected N-deplete host, and importantly, dodder mediates N-systemic signaling between the N-replete and N-deplete hosts bidirectionally. These data indicate that dodder facilitates ecologically meaningful systemic signaling and even transfers nutrients between different host plants and influences host interaction with environmental factors.

A high-quality genome sequence of the dodder *Cuscuta australis* was generated (Sun et al., 2018). It was found that *C. australis* genome experienced a large scale of gene reduction, including important genes involved in photosynthesis, stress resistance, flowering, and circadian regulation, indicating that during the long interaction with host plants, *Cuscuta* lost root and leaf, as well as genes important for regulating root and leaf functions. The genome of *Cuscuta* provides important insight into the evolution of parasitic plants.

Hettenhausen, C., Li, J., Zhuang, H., Sun, H., Xu, Y., Qi, J., Zhang, J., Lei, Y., Qin, Y., Sun, G., Wang, L., Baldwin, I.T., and Wu, J. (2017). Stem parasitic plant *Cuscuta australis* (dodder) transfers herbivory-induced signals among plants. *Proc Natl Acad Sci U S A* 114, E6703-E6709.

Sun, G., Xu, Y., Liu, H., Sun, T., Zhang, J., Hettenhausen, C., Shen, G., Qi, J., Qin, Y., Li, J., Wang, L., Chang, W., Guo, Z., Baldwin, I.T., and Wu, J. (2018). Large-scale gene losses underlie the genome evolution of parasitic plant *Cuscuta australis*. *Nat Commun* 9, 2683.



Monday 1 July 9.00 – 12.40 Host plant resistance

Cuscuta microRNAs target host mRNAs involved in defense and vascular function

Michael J. Axtell

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Parasitic plants can cause serious harm to agriculture. They have unique adaptations that enable their parasitic lifestyles. Stem parasites in the genus *Cuscuta* L. (Family Convolvulaceae) express a unique set of microRNAs at the host-parasite interface. These microRNAs directly target host plant mRNAs involved in defense signaling, vascular system function, and auxin signaling. Using a combination of small RNA sequencing, mRNA sequencing, and 5'-end analysis, we have found a great multitude of such "trans-species" *Cuscuta* microRNAs. *Cuscuta* trans-species microRNAs almost always target highly-conserved, protein-coding regions of host mRNAs. Many *Cuscuta* trans-species microRNAs can be grouped into superfamilies based on shared sequences. Sequence divergence within superfamilies mostly occurs at positions that correspond to synonymous sites of host target mRNAs. A given *Cuscuta* species expresses multiple members of each superfamily. By targeting host mRNA coding regions at highly conserved sites, and expressing multiple microRNA variants to cover any synonymous-site variation, *Cuscuta* trans-species microRNAs may be able to successfully target homologous mRNAs from very diverse hosts. To our knowledge, this is the first clear example of a molecular evolutionary strategy that enables the function of inter-species small RNAs.



Monday 1 July 9.00 – 12.40 Host plant resistance

Interspecific long-distance movement of *Cuscuta* small RNAs control biological processes in host-parasitic plant complex

Subhankar Bera, Kohki Shimizu, Keisuke Tanaka, Shunsuke Yajima, Katsushi Yamaguchi, Shuji Shigenobu, Koh Aoki

Osaka Prefecture University, Japan (SB, KS, KA), NODAI Genome Research Center, Tokyo University of Agriculture, Japan (KT, SY), National Institute for Basic Biology, Japan (KY, SS).

During the interaction of parasitic plants with their host plants, a large number of messenger RNAs and proteins exchange in a bi-directional manner. Recent study on the miRNA movement suggest that accumulation of parasitic plant miRNAs in the interface tissue of host-parasitic complex regulates host gene expression through secondary siRNA production (Shahid et al. 2018). However, movement of small RNAs (sRNAs) beyond interface tissue and their function to regulate the biological processes of respective opponent plants are still unknown. In this study, we analyzed sRNAs in the shoot apical regions and the distant stems of different host-parasitic plant complexes (*Cuscuta japonica*-*Glycine max* and *Cuscuta campestris*-*Arabidopsis thaliana*) by sRNA-seq, and screened the mobile sRNA candidates generated in host and parasitic plants. The results suggested that long-distance mobile sRNAs and their target genes are conserved in two parasitic complexes, implying the conservation of mechanisms in maintaining parasitic relationship between *Cuscuta* and hosts. Interspecific long-distance movement of sRNAs were experimentally verified by stem-loop RT-PCR, and then the effect of mobile sRNAs on the accumulation of target transcripts were examined. In the case of host transcript targeted by sRNAs generated in the *Cuscuta*, accumulation of transcripts such as NIMA (Never In Mitosis /*Aspergillus*) related kinase 3 (NEK3), TPX2-LIKE PROTEIN2 (TPXL2) encoding microtubule associated protein, and FUSCA3 encoding AP2B3-type transcription factor were decreased, and the expression of genes which are controlled in the downstream of these primary target genes were repressed as well. These results demonstrated that sRNAs that moved long-distance from plant to plant regulate biological processes such as cell cycle progression and cytoskeletal organization of the opponent plants. This work was partly supported by the Cooperative Research Grant of the Genome Research for Bio-Resource (Tokyo University of Agriculture), the NIBB Cooperative Research Program (17-429) and KAKENHI (No. 18H03950).

Shahid, S., G. Kim, N.R. Johnson, E. Wafula, F. Wang, C. Coruh, V. Bernal-Galeano, T. Phifer, C.W. dePamphilis, J.H. Westwood, and M.J. Axtell, 2018. MicroRNAs from the parasitic plant *Cuscuta campestris* target host messenger RNAs. *Nature* 553:82-85



Monday 1 July 9.00 – 12.40 Host plant resistance

A peptide motif of a parasitic plant cell wall protein is recognized by the receptor protein CuRe1 and induces defense in tomato

Volker Hegenauer (1), Max Karner (1), Julien-Alexander Hollmann (2), Peter Slaby (1), Frank Menke (3), Ronja Burggraf (1), Kirsten Krause (2), Mark Stahl (1) and Markus Albert (1)

(1) *University of Tübingen, ZMBP - Plantbiochemistry, 72076 Tübingen, Germany*; (2) *The Arctic University of Norway, Tromsø, Norway*; (3) *The Sainsbury Laboratory, Norwich, UK*

Plant species of the genus *Cuscuta* are holoparasites which infect both dicotyledonous and monocotyledonous host plants. One notable exception is tomato, which is specifically resistant to *Cuscuta reflexa*. We discovered that tomato responds to a small proteinaceous factor occurring exclusively in various *Cuscuta* spp. with plant immune responses typically activated after the recognition of microbe-associated molecular patterns (MAMPs). We identified the tomato cell surface receptor-like protein CUSCUTA RECEPTOR 1 (CuRe1) as a critical component of the perception system for the parasite-associated molecular pattern. CuRe1 is sufficient to confer responsiveness to this *Cuscuta* factor and increased resistance to parasitic *C. reflexa* when heterologously expressed in otherwise susceptible host plants, such as *Nicotiana benthamiana*. Recently, we deciphered the protein sequence of the parasitic molecular pattern and cloned the corresponding gene of *Cuscuta*. The minimal peptide motif that is recognized by CuRe1 consists of 21 amino acid residues and originates from a 11 kDa cell wall protein of yet unknown function. In general, our results underline that some plants are able to recognize parasitic plants as non-self via molecular patterns as it is usually known for the perception of microbial pathogens. These findings thus provide potential for engineering resistance to parasitic plants in crops.



Monday 1 July 9.00 – 12.40 Host plant resistance

Molecular basis for tomato resistance to the parasitic plant *Cuscuta*

Neelima Sinha, Min-Yao Jhu, Moran Farhi, Li Wang, Richard Philbrook

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Parasitic angiosperms directly attach to host plants using specialized organs known as haustoria, which function as physiological bridges to extract nutrients and water from their hosts. *Cuscuta* species (dodders) are common and agriculturally destructive flowering stem parasitic plants. Many *Cuscuta* species are listed in the Federal or State Noxious Weed lists, including *Cuscuta pentagona* (*C. pentagona*). Reports have shown a 50–72% reduction in tomato yield due to *Cuscuta*. Because of the intimate physiological connection between host plants and parasites, most traditional herbicides and control methods have not been effective or are too costly. We used transcriptomics to identify genes upregulated in *Cuscuta* upon attachment to host. Expression of key upregulated genes was reduced using host-induced-gene-silencing and haustorium formation monitored. Reduction in expression of some of the identified genes attenuated parasitism. While most tomato cultivars can be parasitized by *C. pentagona*, we analyzed Heinz hybrid cultivars, which exhibited resistance to dodders. Local lignification in the stem cortex upon dodder attachment led to resistance to haustorium penetration in the resistant cultivars. Key resistance genes included an AP2-like transcription factor, a MYB transcription factor and an NBS-LRR (a gene encoding a nucleotide-binding site leucine-rich repeat protein). The involvement of these and other genes in inducing local lignification was deciphered using a combination of CRISPR and virus based gene expression. The results of this study may help develop a parasite-resistant system in crops to reduce economic losses in agriculture.



Monday 1 July 9.00 – 12.40 Host plant resistance

Characterization of resistance to sunflower broomrape (*Orobanche cumana* W.) in sunflower (*Helianthus annuus* L.)

Dana Sisou, Yaakov Tadmor and Hanan Eizenberg

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Orobanche spp. (broomrapes) are chlorophyll-lacking obligate root parasites, drawing all required water and nutrition from the host. Sunflower broomrape (*Orobanche cumana* Wallr.) is regarded as one of the most important production constraints of sunflower in many countries in Europe and the Mediterranean region. Breeding for resistance is the most effective and beneficial method to manage sunflower broomrape infestation. However, the resistance of new cultivars is often overcome. Therefore a better knowledge of the mechanisms responsible for resistance to parasitic plants is necessary to improve the production of crops with long-lasting resistance. The aim of this study was to elucidate the broomrape resistance mechanism of the sunflower variety EMEK3 (a confectionary sunflower hybrid with high and long-term resistance to sunflower's broomrape). Observations of host-parasite interactions along with histological sections of incompatible interaction of *O. cumana* on the resistant variety EMEK3 showed that the parasite intrusive cells are stopped in the host cortex, during the penetration attempt, and before reaching the endodermis, indicating a Pre-Haustorial mechanism of resistance. Total RNA was extracted from EMEK3 roots and from roots bulks of other *O. cumana* resistant and susceptible sunflower lines during the resistance response. A comparative RNA-sequencing identified differentially expressed genes (DEG) associated with the resistance mechanism. The preliminary results of the DEG analysis revealed 3 differentially expressed genes communal to the resistant bulk and EMEK3: β -1,3-endoglucanase, β -glucanase 40-like and ethylene-responsive transcription factor 4-like. These genes are known as PR (Pathogen Related) in other plant species. To conclude, according to the present results, we can presume that the resistant mechanism of EMEK3 is based on the lack of capability of the broomrape to intrude the host root tissue thus the resistance response constitute a physical barrier to prevent parasite penetration.



Monday 1 July 14.15 – 18.00 Ecology, phylogeny and evolution

Eco-evolutionary causes and consequences of parasitism in plants

Susann Wicke

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Parasitism is a successful way of life, one that is ubiquitous to all organismal domains. In plants, the transition from a free-living organism to being a parasite that steals water and nutrients either from another plant or from mycorrhizal networks provides a significant ecological-evolutionary advantage in highly competitive and resource-poor environments. The transition of plants to a nonphotosynthetic lifestyle brings about extreme morphological changes and massive genetic reconfigurations. Here, we explore the progression of the so-called parasitic reduction syndrome on the genetic level and elucidate how parasitism affects diversification patterns. The natural diversity of parasitic specializations and phylostatistical hypothesis testing revealed that already the establishment of obligate parasitism triggers genomic reconfigurations. After the eventual loss of photosynthesis, functional and physical reduction proceed rapidly predominantly in plastid genomes, accompanied by a gradual acceleration of molecular evolutionary rates. The transition to obligate parasitism apparently triggers an eco-evolutionary feedback loop that affects all genomic compartments, which might further trophic specialization (Wicke et al. 2013, 2016). As this happens, we observe that diversification patterns change en route to holoparasitism. We hypothesize that parasite speciation and diversification is co-shaped by an increasing dependency on their host plants, which creates an isolating barrier absent from ordinary plants (Maul et al. 2018).

- Maul, K., M. Krug, D.L. Nickrent, K.F. Müller, D. Quandt, and S. Wicke. 2018. "Morphology, geographic distribution, and host preferences are poor predictors of phylogenetic relatedness in the mistletoe genus *Viscum* L." *Molecular Phylogenetics and Evolution* 131: 106–15.
- Wicke, S., K.F. Müller, C.W. dePamphilis, D. Quandt, S. Bellot, and G.M. Schneeweiss. 2016. Mechanistic model of evolutionary rate variation en route to a nonphotosynthetic lifestyle in plants." *Proceedings of the National Academy of Sciences of the United States of America* 113: 9045–50.
- Wicke, S., K.F. Müller, C.W. dePamphilis, D. Quandt, N.J. Wickett, Y. Zhang, S.S. Renner, and G.M. Schneeweiss. 2013. "Mechanisms of functional and physical genome reduction in photosynthetic and non-photosynthetic parasitic plants of the Broomrape family." *The Plant Cell* 25: 3711–25.



Monday 1 July 14.15 – 18.00 Ecology, phylogeny and evolution

A neglected alliance in battles against parasitic plants: AM and rhizobial symbioses alleviate damage to a legume host by root hemiparasitic *Pedicularis* species

Xiao-Lin Sui, Ting Zhang, Yu-Qing Tian, Rui-Juan Xue, Ai-Rong Li

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Despite their ubiquitous distribution and significant ecological roles, soil microorganisms have long been neglected in investigations addressing parasitic plant-host interactions. Because nutrient deprivation is a primary cause of host damage by parasitic plants, we hypothesized that beneficial soil microorganisms conferring nutrient benefits to parasitized hosts may play important roles in alleviating damage. We conducted a pot cultivation experiment to test the inoculation effect of an arbuscular mycorrhizal (AM) fungus (*Glomus mosseae*), a rhizobium (*Rhizobium leguminosarum*) and their interactive effects, on alleviation of damage to a legume host (*Trifolium repens*) by two root hemiparasitic plants with different nutrient requirements (N-demanding *Pedicularis rex* and P-demanding *P. tricolor*). Strong interactive effects between inoculation regimes and hemiparasite identity were observed. The relative benefits of microbial inoculation were related to hemiparasite nutrient requirements. Dual inoculation with the rhizobium strongly enhanced promotional AM effects on hosts parasitized by *P. rex*, but reduced the AM promotion on hosts parasitized by *P. tricolor*. Our results demonstrate substantial contribution of AM and rhizobial symbioses to alleviating damage to the legume host by root hemiparasites, and suggest that soil microorganisms are critical factors regulating host-parasite interactions and should be taken into account in future studies.

- Li, A.R., K.Y. Guan, R. Stonor, S.E. Smith and F.A. Smith, 2013. Direct and indirect influences of arbuscular mycorrhizal fungi on phosphorus uptake by two root hemiparasitic *Pedicularis* species: Do the fungal partners matter at low colonization levels? *Annals of Botany* 112: 1089–1098.
- Li, A.R., Y.J. Li, S.E. Smith, F.A. Smith and K.Y. Guan, 2013. 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. *Annals of Botany* 112: 1099–1106.
- Li, A.R., F.A. Smith, S.E. Smith and K.Y. Guan, 2012. Two sympatric root hemiparasitic *Pedicularis* species differ in host dependency and selectivity under phosphorus limitation. *Functional Plant Biology* 39: 784–794.
- Sui, X.L., A.R. Li, Y. Chen, K.Y. Guan, L. Zhuo and Y.Y. Liu, 2014. Arbuscular mycorrhizal fungi: potential biocontrol agents against the damaging root hemiparasite *Pedicularis kansuensis*? *Mycorrhiza* 24: 187–195.
- Sui, X.L., T. Zhang, Y.Q. Tian, R.J. Xue and A.R. Li, 2019. A neglected alliance in battles against parasitic plants: arbuscular mycorrhizal and rhizobial symbioses alleviate damage to a legume host by root hemiparasitic *Pedicularis* species. *New Phytologist* 221: 470–481.



Monday 1 July 14.15 – 18.00 Ecology, phylogeny and evolution

When the same is not the same

Peter Toth, Anna Katarzyna Undas and Harro Bouwmeester

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The broomrapes, *Orobanche* spp. and *Phelipanche* spp. (Orobanchaceae), are a taxonomically complicated group of holoparasitic flowering plants with significant impact on ecosystems and host plant performance. From their flowers, they emit a plethora of volatile organic compounds (VOCs), which act as a detailed language for communication with the surrounding environment. These VOCs support phylogenetic relationships within the Orobanchaceae and their taxonomy. In this study we investigated if VOCs of single broomrape species are always the same irrespective of the region and habitat, host plant species or origin of the seeds. Dynamic headspace sampling was performed either in the greenhouse, headspace chamber or in the field. All headspace samples were in turn analyzed using GC-MS. Significantly different metabolites were used for further analysis using unsupervised PCA. Two species with contrasting host range, *Orobanche flava* (narrow) and *Phelipanche ramosa* (broad) were used as model. The host of naturally growing *O. flava* was *Petasites* spp. (butterbur) on 4 geographically remote sites in Slovakia. *Ph. ramosa* (Slovak and Italian origin) was grown in the greenhouse under the same conditions on various hosts (*Arabidopsis*, cabbage, oilseed rape, tobacco, tomato), including three different tomato varieties. The global separation of the broomrapes based on VOCs (recorded during the previous experiments) was consistent and precise. Nevertheless, closer inspection of the VOC data revealed marked differences in the VOC profile between locations, host species and even varieties in the case of tomato. The results suggest that there are not only differences in the VOCs (language) between broomrape species, but there are probably also different slangs (chemotypes), as a result of location and host. We hypothesise that one of the possible ecological explanations of a small change in VOC composition could be an adaptation of broomrapes to local pollinators. The results will be discussed and ecological implications highlighted.



Monday 1 July 14.15 – 18.00 Ecology, phylogeny and evolution

Genetic diversity of *Orobanche cumana* (sunflower broomrape) populations at the world level revealed by SSR markers

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Orobanche cumana (sunflower broomrape) is a holoparasitic plant which lacks photosynthetic activity, parasitizing roots of sunflower (*Helianthus annuus*). The *O. cumana* populations which emerged in China and in France still remain unwell characterized. In order to study their genetic diversity and structure within and between populations, Five populations (259 individuals) from China and 19 populations (103 individuals) from France were genotyped using 20 SSR markers and compared to populations from Spain, Bulgaria and Turkey. The genetic diversity between populations enabled to differentiate genetic pools, clustered according to their geographical location at the world level. We clearly distinguished Chinese and French populations as being new genetic pools compared to the other tested European populations. The Chinese populations were clustered in two genetic pools from the Xinjiang (XJ) region close to Kazakhstan and Russia and from Inner Mongolia in North of China. The phylogenetic tree was consistent with principle component analysis (PCA), and suggested that the XJ population from China was the most divergent population. We found a high number of private alleles in the XJ population and population genetic parameters indicated an independent evolution in XJ region. Finally, we found a low genetic diversity within each population. All results will be detailed and hypothesis that could affect the adaption and evolution process of *O. cumana* populations will be discussed.

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Monday 1 July 14.15 – 18.00 Ecology, phylogeny and evolution

Facultative parasitism: an evolutionary precursor of complete parasitism or an effective strategy in its own right?

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Parasitism in plants is developed to varying degrees, as reflected in host plant dependency. From an evolutionary perspective, obligate parasites are most advanced, as parasitism is developed to the extent that they completely rely on a host (Westwood et al., 2010). Consequently, seeds of most obligate parasites require exposure to host signal molecules for germination, allowing them to synchronize with their host. Facultative parasites are less evolved. In absence of a host, they complete their life cycle on their own, and only with a host plant in reach, they parasitize. In rain-fed rice production systems, a sharp demarcation was noted between parasite occurrence in two ecosystems (Kabiri et al., 2015). Under upland conditions, obligate hemi-parasitic weeds thrive. In contrast, rain-fed lowlands harbour *Rhamphicarpa fistulosa*, a facultative parasitic weed (Rodenburg et al., 2015). Whether dominance of *R. fistulosa* in this wetland ecosystem is linked to the more opportunistic life-history strategy of facultative parasites was investigated. Additionally, the study examined if specific life-cycle traits are accountable for the apparent close fit between parasite and ecosystem. To this end, two versions of a life-cycle-stage population model, differing in seed germination response, were developed. In the obligate model, seed germination solely occurs near a host, whereas in the facultative model germination is independent of host plant presence. In this facultative model, seed production rate is coupled to attachment status of the parasite, to account for the lower seed production in the absence of parasitism (Kabiri et al., 2016). Model input parameters related to relevant life-cycle processes (e.g. germination, seed mortality, seed production) were systematically varied, after which simulations were conducted to determine weed seed density in equilibrium. The ratio of the simulated equilibrium densities obtained with both models was used to express the relative success of the two strategies. Comparison revealed that neither the obligate, nor the facultative strategy consistently reached the highest equilibrium density, rather the result depended on model input parameters. Analysis further showed that the difference between strategies was more distinct if simulations presumed lower host plant abundance. For the specific case of *R. fistulosa* under lowland conditions, seed longevity was identified as a key trait. The short seed longevity, stemming from alternate wetting and drying of lowland soils, rendered a facultative life-cycle strategy more successful than an obligate strategy. Facultative parasitism should thus be regarded a strategy in its own right and not just a precursor of complete parasitism.

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Monday 1 July 14.15 – 18.00 Ecology, phylogeny and evolution

Fitness of reciprocal F1 hybrids between *Rhinanthus minor* and *R. major*

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The performance of first-generation hybrids determines to a large extent the long-term outcome of hybridization in natural populations. F1 hybrids can facilitate further gene flow between the two parental species, especially in animal-pollinated flowering plants. We studied the performance of reciprocal F1 hybrids between *Rhinanthus minor* and *R. major*, two hemiparasitic, annual, self-compatible plant species, from seed germination to seed production under controlled conditions and in the field. We sowed seeds with known ancestry outdoors before winter and followed the complete life cycle until plant death in July the following season. Germination under laboratory conditions was much lower for the F1 hybrid formed on *R. major* compared to the reciprocal hybrid formed on *R. minor*, and this confirmed previous results from similar experiments. However, this difference was not found under field conditions, which seems to indicate that the experimental conditions used for germination in the lab are not representative for the germination behaviour of the hybrids under more natural conditions. The earlier interpretation that F1 hybrid seeds formed on *R. major* face intrinsic genetic incompatibilities therefore appears to be incorrect. Both F1 hybrids performed as well as or sometimes better than *R. minor*, which had a higher fitness than *R. major* in one of the two years in the greenhouse and in the field transplant experiment. The high fitness of the F1 hybrids confirm findings from naturally mixed populations, where F1 hybrids appear in the first year after the two species meet and which leads to extensive advanced-hybrid formation and introgression in subsequent generations.

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Tuesday 2 July 9.00 – 12.45 Genes and genomes

Cuscuta campestris: A plant genome under the influence of a parasitic lifestyle

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The parasitic genus *Cuscuta* (Convolvulaceae) has evolved a unique anatomy boasting such conspicuous morphological abnormalities as a lack of roots and the reduction of leaves and cotyledons to microscopic scales. In contrast to photosynthetically active non-parasitic plants, *Cuscuta* has only limited need for photosynthesis and an ensuing reduced or absent capacity for autotrophic growth, ostensibly leading to a different selection pressure on the parasite's genomes. While the chloroplast genome has been intensively studied in the past, the analysis of the other cellular genomes has lagged significantly behind.

With the publication of the first parasitic plant genomes in 2018 (*Cuscuta campestris* by Vogel et al. and *C. australis* by Sun et al.), it has finally become possible to investigate what effect the morphological and physiological reductions have had on their overall coding capacity. An emerging hallmark of *Cuscuta*'s genomes are losses of genes performing redundant or obsolete functions. In particular, nuclear subunits of key plastid complexes have been affected by deterioration, but other processes connected, for example, to root functions have been affected by these losses, too.

Despite these gene losses, *C. campestris* boasts more than 44000 predicted gene loci. In part, this number is accounted for by a whole genome duplication event some 1-2 million years ago that has been accompanied by an amplification wave of retrotransposon elements. In addition, new genes were gained by trans-species horizontal gene transfer from the genomes of their numerous host plants, while endogenous gene transfer from the organellar genomes to the nucleus appears to be limited.

An important group of genes codes for proteins that are dually targeted to the nucleus and the chloroplasts and that can be involved in intracellular signaling and coordination of gene expression in the different compartments. Comparing the players that control intracellular communication will give us more insight into these important processes.



Tuesday 2 July 9.00 – 12.45 Genes and genomes

Genetic basis for host and parasitic plant communication

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The Orobanchaceae family consists of root parasitic plants except one genus *Lindenbergia*. This family contains various ranges of root parasitic plants from facultative parasites to obligate parasites, including the world most devastating pests *Striga*, *Orobanche* and *Phelipanche* spp. The life cycles of these obligate parasitic weeds are highly dependent on their hosts; the seeds germinate in response to host-derived strigolactones, their radicle tips deform and develop terminal haustoria, and shoot elongation occurs after successful host parasitization. On the other hand, facultative parasites, such as *Triphysaria* and *Phtheirospermum* spp, do not require strigolactones for their germination, have their own root system, and form lateral haustoria on their roots when host roots are nearby present. The Orobanchaceae family has probably acquired root parasitism once during their evolution, and obligate parasites were evolved from facultative parasites. However, the genetic and genomic basis for their evolution are not well understood.

We have sequenced the genomes of an obligate parasitic plant *Striga asiatica* and a facultative parasitic plant *Phtheirospermum japonicum*. Evolutional analyses of gene families revealed the expansion of parasitism related genes. Genes encoding strigolactone receptors are highly duplicated in the *Striga* genome mainly via tandem duplication. *Striga* genome acquired a large genomic region from Poaceae host species via horizontal gene transfers. Comparative genomic analysis implies the events occurred during evolution of plant parasitism in Orobanchaceae. Moreover, we have developed forward genetic tools using *P. japonicum* as a model plant. Our screening identified several mutants, which have defects in haustorium formation and morphology. Phenotypic analysis told us genetic programs underlying plant parasitism.



Tuesday 2 July 9.00 – 12.45 Genes and genomes

Exploring the evolutionary origin of haustorium development in root parasitic plants

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The Orobanchaceae root parasitic plants develop haustoria in response to host root exudates. Similar mechanisms are proposed to regulate the interactions of non-parasitic plant roots as well [1]. Changes in cis-regulatory elements are thought to contribute to phenotypic divergence more than protein changes because of the higher pleiotropic effects associated with altering the coding sequence [2]. Therefore, we hypothesize haustorium development may have been co-opted to an earlier root-root recognition mechanism through changes in the cis-element regulation of autotrophic plant genes and pathways. To investigate this, I am comparing the promoters of haustoria related genes from Orobanchaceae parasites to identify cis-elements regulating the transcriptional changes in response to host root exudate. We created an RNASeq dataset for the first 24 hours of haustorium development in the hemiparasite *Triphysaria versicolor* to identify groups of genes co-expressed during haustorium initiation and early development. Promoters for these genes were obtained through sequencing the *T. versicolor* genome using a 10X Genomics library preparation and the Illumina NovaSeq platform. The promoters of genes with similar transcriptional changes are being compared for over-represented cis-elements that may be responsible for their co-regulation. In addition to this approach, we are using phylogenetic footprinting to identify cis-elements conserved between Orobanchaceae species. We collected eighteen species of annual hemiparasitic Orobanchaceae and are comparing the promoters of orthologous genes with similar host induced expression to find conserved cis-elements. The cis-elements identified through both methods are being assayed for host induced spatio-temporal activity using a fluorescent protein system. By characterizing the transcriptional regulation of genes involved in haustorium initiation and early development in Orobanchaceae parasites, it can identify the changes needed for haustoria to originate from a non-parasitic plant.

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Tuesday 2 July 9.00 – 12.45 Genes and genomes

Where the Action Is: Gene Expression at the Parasite-Host Interface

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Orobanchaceae is the only plant family with members representing the full spectrum of parasitic lifestyles including a non-parasitic sister lineage. This diverse parasitic family was leveraged by the Parasitic Plant Genome Project to discover core parasitism genes through comparative transcriptomics. In this study three parasitic plants, *Triphysaria versicolor*, *Striga hermonthica*, and *Phelipanche aegyptiaca*, were grown on their respective hosts and haustoria were collected in triplicate. In contrast to previous studies, the haustoria were dissected via laser capture microdissection (LCM). LCM allowed the entire haustoria to be dissected into three parts: the host tissue, the interface cells, and the parasite tissue. RNAseq libraries were generated and analyzed for the host tissue, interface, and parasite tissue. By isolating the individual tissues, gene expression can be observed at the interface, where the most direct interactions occur between host and parasite. Here through DESeq2, the difference between parasite body and the interface have been examined. Differential expression analyses indicate up-regulation at the parasite-host interface of genes encoding proteins involved in cell wall processing and root hair development. The parasite body, however, shows different expression patterns from the interface including upregulation of stress-related genes and an abundance of transporters. In addition to the parasite expression, this dataset has given insights into host plant gene expression in the haustorium. Gene Ontology (GO) analysis of host side haustorial tissue reveals an underrepresentation of defense-related genes compared to the control (unparasitized) host tissue, indicating the parasite may be reducing the host defense response during parasitism.



Tuesday 2 July 9.00 – 12.45 Genes and genomes

Convergent horizontal gene transfer and crosstalk of mobile nucleic acids in parasitic plants

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Horizontal gene transfer (HGT, the movement and genomic integration of DNA across species boundaries, is a common mechanism used by bacteria to acquire novel traits such as antibiotic resistance but is far less common in plants and other eukaryotes. Although instances of HGT have been documented among autotrophic plants, the process more frequently involves parasitic plants, likely due to their intimate feeding connections with their host plants. However, the extent of HGT, the mechanisms of transfer, and functional implications of the acquired sequences remain unclear. Here, in the stem parasite *Cuscuta*, we identify 108 transcribed and likely functional HGT (fHGT) events in *Cuscuta campestris* and related species, more than twice as many as found in a detailed examination of Orobanchaceae, a family of root parasitic plants. Except for genes encoding cell-wall modifying enzymes, which were captured by fHGT in *Cuscuta* but not Orobanchaceae, surprisingly similar sets of genes were acquired independently by the two lineages, and the majority are expressed highly in the haustorial feeding structures, suggesting selective retention of HGTs and an adaptive role for specific additional genes in parasite biology. 16-20 of the transcribed HGT events are inferred as ancestral in *Cuscuta* based on transcriptome sequences from species across the phylogenetic range of the genus, implicating fHGT in the successful radiation of *Cuscuta* parasites. Genome sequencing of *C. campestris* supports DNA - rather than RNA - transfer as the mechanism of fHGT. Many of the *C. campestris* horizontally acquired genes are also frequent sources of 24 nt small RNAs that are typically associated with RNA-directed DNA methylation. One HGT encoding a leucine-rich repeat protein kinase overlaps with a microRNA that has been shown to regulate host gene expression, suggesting that HGT-derived parasite small RNAs may function in the parasite-host interaction. This study enriches our understanding of HGT by describing a parasite-host system with unprecedented gene exchange that points to convergent evolution of fHGT events and the functional significance of horizontally transferred coding and noncoding sequences.

Zhenzhen Yang, Eric Wafula, Gunjune Kim, Saima Shahid, Joel R. McNeal, Paula E. Ralph, Prakash R. Timilsena, Wen-bin Yu, Elizabeth A. Kelly, Huiting Zhang, Thomas Nate Person, Naomi S. Altman, Michael J. Axtell, James H. Westwood, Claude W. dePamphilis. Convergent horizontal gene transfer and crosstalk of mobile nucleic acids in parasitic plants. *Nature Plants* (in press).



Tuesday 2 July 9.00 – 12.45 Genes and genomes

The identification of candidate pathogenicity-related genes from the genome of *Striga hermonthica*

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Plant parasites have evolved suites of pathogenicity-related genes that interact with host biology to either facilitate host colonization or trigger host defense responses. *Striga hermonthica* is a root parasitic plant that causes severe yield loss in agriculturally-important cereal crops grown throughout Sub-Saharan Africa. In order to develop a sustainable, integrated control strategy against *S. hermonthica*, a better knowledge of pathogenicity-related genes is desired. Despite this, and in contrast with other plant parasites such as fungi and nematodes, very little is known about the nature of pathogenicity-related genes in parasitic plants.

This work aimed to investigate the whole-genome complements of pathogenicity-related genes in *S. hermonthica*. First, a whole-genome sequence was assembled and annotated for a single *S. hermonthica* individual. This genome was then mined using an in silico approach to select genes encoding putatively-secreted proteins, as these have the potential to be delivered from the parasite into the host plant, and thus may play a role in the host-parasite interaction. A detailed investigation of the *S. hermonthica* secretome revealed a large number of small proteins that were rich in cysteine amino acid residues; which is a hallmark of pathogenicity-related proteins in other phytoparasite systems. The secretome was enriched with a range of protein domains, including those involved in cell wall modification (e.g. pectinesterase and xyloglucan endo-transglycosylase domains) and protease activity (e.g. papain cysteine protease and subtilase domains). Many of these domains were also enriched to a greater extent than was found in an identical analysis carried out for the closely-related, non-parasitic plant, *Mimulus guttatus*.

This work has provided a genome-wide insight into the suites of putatively-secreted proteins that have the potential to play a role in the *S. hermonthica*-host plant interaction and has facilitated the prioritisation of candidates for future functional studies. I will present an overview of the findings and highlight specific examples of candidate pathogenicity-related genes that are biologically interesting in the context of a *S. hermonthica*-host plant interaction.



Tuesday 2 July 9.00 – 12.45 Genes and genomes

Lack of evidence for horizontally transferred genes in mitochondria of *Cuscuta* species

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Parasitic plants grow in direct contact with their hosts, providing an increased opportunity for transfer of genetic material between them. This horizontal gene transfer (HGT) has been demonstrated for a number of parasitic angiosperms, with movement of genes from both hosts into parasites and parasites into hosts. Although the extent of HGT involving the nuclear genome is still being explored, so far HGT seems to be relatively common for mitochondrial genes. Some hosts of the parasitic angiosperm *Cuscuta* have been shown to retain copies of mitochondrial genes similar to those of the parasite, suggesting that HGT from parasite to host has occurred multiple times. Recent nuclear genomic work in *Cuscuta campestris* has also identified numerous HGT candidates, suggesting transfers occur in the opposite direction as well. Using published long- and short-read sequencing data sets, we assembled the mitochondrial genomes of two *Cuscuta* species (*C. campestris* and *C. australis*) and examined gene content to assess the extent of HGT. We conducted phylogenetic analyses for each annotated gene in our assembled mitochondria along with publicly available copies from *Cuscuta gronovii* and 19 representative taxa from across angiosperms, including *Ipomoea*, a close relative of *Cuscuta*. Surprisingly, in most cases, mitochondrial genes from the *Cuscuta* species were well supported as grouping together and as sister to those of *Ipomoea*, suggesting vertical rather than horizontal acquisition. No strongly supported groupings were found in which the *Cuscuta* genes were sister to distantly-related taxa. Our results suggest that *Cuscuta* species have not retained mitochondrial HGT copies from their hosts, despite earlier evidence for HGT in the other direction. It is unclear whether the relatively small sizes of the *Cuscuta* mitochondrial genomes we assembled (~280 kb) are related to this apparent lack of horizontally-acquired genes.



Tuesday 2 July 14.15 – 18.10 Molecules and Biochemistry

CHEMICALS that control *Striga* germination

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Chemicals that control parasitism of root parasitic weeds will be very useful to improve crop productivity. The first stage of the parasitism of *Striga hermonthica* is the seed germination, which are triggered by strigolactones (SLs), cytokinins (CKs) or ethylene (ET), so several approaches can be used to develop chemically synthesized function regulators of these hormones. As suicidal germination inducers have been thought as an idealistic strategy for *Striga* infestation, several SL agonists has been developed and their efficacy as suicidal germination inducers was reported. As well as these SL agonists, agonists of CKs or ET can be hopeful candidate chemicals for suicidal germination inducers which stimulate germination of *Striga* seeds without conditioning. Chemicals that control exudation of these hormones from host roots can be good chemical tools to reduce the damage by *Striga* such as SL biosynthesis inhibitors and SL transport inhibitors in host plants.

In addition, chemicals that control not only perception of SLs, CKs and ET, but also biosynthesis of CKs and ET in root parasitic weeds can be used as chemical tools to control *Striga* infestation. Here, on the basis of the targets of chemicals both in hosts and root parasitic weeds as noted above, the recent results in our laboratory will be shown.

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Tuesday 2 July 14.15 – 18.10 Molecules and Biochemistry

Signalling pathways in *Striga hermonthica* germination

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In Africa, the parasitic weed, *Striga hermonthica*, infects major food crops which results in devastating yield losses for over 100 million subsistence farmers. Because *Striga* is an obligate parasite, it is essential for *Striga* seed to germinate in the vicinity of a plant host. Roots of hosts exude the hormone, strigolactones (SLs), which are perceived by *Striga* seed to indicate that a host is nearby and thereby germinate. In higher plants, various signals like hormones, light and nutrients are transduced by signaling pathways into combinations of gene expression which compose a “germination code”. *Striga*, however, has modified its response to signals that would typically promote germination of seeds from non-parasitic plants. Instead, *Striga* has evolved high sensitivity to SLs. To elucidate the molecular mechanisms underpinning *Striga* germination, the Lumba lab investigates how SLs are perceived and transduced by signalling components from *Striga*. We have applied three strategies to study a non-model system like *Striga*: (1) heterologous expression of *Striga* genes in *Arabidopsis* to analyze function; (2) transcriptomic analyses of germination and; (3) biochemical approaches including large-scale yeast two-hybrid methods to identify interactions among *Striga* proteins. As a resource for the community, we have developed a *Striga hermonthica* eFP browser which is an interactive tool for visualizing gene expression data from the Parasitic Plant Genome Project (PPGP) database. This data visualization tool enables researchers to explore the expression levels of *Striga* genes at various stages of development.

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Tuesday 2 July 14.15 – 18.10 Molecules and Biochemistry

Complementary Hormone-Based Approaches for Striga Control

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The root parasitic plant *Striga hermonthica* is a major threat for global food security, devastating cereals yields particularly in sub-Saharan Africa. *Striga* is an obligate parasite that entirely depends on a suitable host to survive and complete its lifecycle, and relies on host-derived signals, mainly strigolactones (SLs), for seed germination. SLs are carotenoid-derived plant hormone synthesized via the central intermediate carlactone. Recently, we have developed several carlactonoic acid-based SL analogs and tested them with respect to different SL activity, including lethal induction of *Striga* seed germination in the absence of a host. To realize this strategy, called suicidal germination, in infested fields of sub-Saharan Africa, we have also established an efficient application protocol for rain-fed agriculture that prevails in this region. Application of this protocol led to significant decrease in *Striga* infestation in infested farmers fields in Burkina Faso. In *Striga* seeds, SLs are perceived by the family of *Striga hermonthica* HYPOSENSITIVE to LIGHT (ShHTL) receptors. We have elucidated the structure of ShHTL7, the most sensitive SL receptor in *Striga* seeds, and identified the common detergent Triton X-100 as an SL antagonist. High-resolution X-ray structures show that Triton X-100 specifically plugs the catalytic pocket of this *Striga* receptor. Application of Triton X-100 inhibits *Striga* seed germination, opening up the possibility of using it as a lead compound to develop specific *Striga* germination inhibitors that can be applied in hostTMs presence and can complement the suicidal germination strategy. In a further approach, we discovered a novel carotenoid-derived regulatory metabolite, zaxinone, which is required for normal rice growth and development. In addition, this hormone-like metabolite acts as growth promoting compound and as a negative regulator of SL biosynthesis and release. Application of zaxinone in pot experiments showed that it alleviates *Striga* infestation. We are currently developing zaxinone mimics that can be used to accelerate growth and reduce SL release, thus helping cereal crops to escape *Striga* infestation. In this talk, we will give an overview about these complementary, hormone-based approaches for combating *Striga* and present obtained results.

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Tuesday 2 July 14.15 – 18.10 Molecules and Biochemistry

Unravel strigolactone signaling and controlling parasitic plant behaviors in *Striga*

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Striga hermonthica (*Striga*) parasitizes crops widely across various parts of sub-Saharan Africa, causing loss in crop yields that result in economic pressure on millions of smallholder farmers and lead to annual losses of billions of dollars. As *Striga* seeds require host-generated strigolactones (SLs) to germinate, understanding the mechanism of SL signaling could lead the development of chemical agent for controlling these noxious weeds. We have been approaching to the problem with small molecule probes including a fluorogenic probe for SL receptor called yoshimulactone green which allowed us to identify the 11 members of SL receptors which orchestrate a dynamic wave-like pattern of SL perception in *Striga* seeds (Tsuchiya and Yoshimura et al., *Science*, 2015). The discovery was recently extended to develop a femto-molar range germination stimulants for *Striga* (Uraguchi et al., *Science*, 2018). In this seminar, I will present how chemical biology approach contributes to understanding this serious problem and developing lead compounds to combat against *Striga*.

Uraguchi, D., Kuwata, K., Hijikata, Y., Yamaguchi, R., Imaizumi, H., AM, S., Rakers, C., Mori, N., Akiyama, K., Irle, S., McCourt, P., Kinoshita, T., Ooi, T., and Tsuchiya, Y. (2018). A femtomolar-range suicide germination stimulant for the parasitic plant *Striga hermonthica*. *Science*, 362, 1301–1305.

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Tuesday 2 July 14.15 – 18.10 Molecules and Biochemistry

Structural and biochemical characterization of strigolactone parasitic receptors, understanding their functionality and how to inhibit them.

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Striga hermonthica is the most destructive food crop parasite in the developing world. It invades its hosts by perceiving a group of host-derived small molecules called strigolactones. The receptors in charge of sensing strigolactones are encoded by a group of a/b hydrolases that not only recognize strigolactones but also hydrolyze them. The mechanisms of how SL receptors perceive their ligands and the role of hydrolysis in this perception are still rudimentary explained. To this end, I have crystallized three a/b hydrolases, two from parasitic plants in complex with inhibitory compounds (ShHTL7 and ShHTL9) and one from *Arabidopsis* (DLK2). Using these structures, I carried out molecular dynamics (MD) simulations, which led us to hypothesize about the role of hydrolysis and conformational change in protein signalling. At the same time, I have used the crystallography information obtained to determine the cause for hypersensitivity of some strigolactone receptors, creating hybrid proteins with specific hydrolytic abilities. Ultimately, the data generated will aid in the development of strigolactone agonists and antagonists that perturb the germination of the parasite *Striga hermonthica*.

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- Xu, Y., Miyakawa, T., Nakamura, H., Nakamura, A., Imamura, Y., Asami, T., Fernández-Aparicio, M. (2016). Structural basis of unique ligand specificity of KAI2-like protein from parasitic weed *Striga hermonthica*. *Scientific Reports*, 6, 31386.



Tuesday 2 July 14.15 – 18.10 Molecules and Biochemistry

Identification and characterization of β -galactosidase capable of hydrolyzing planteose in *Orobanche minor* as a target for control of root parasitic weeds

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Root parasitic weeds depend on the assimilated carbon by the photosynthesis of the hosts. The dry seeds of root parasitic weeds contain only small amounts of storage substances, as the assimilated carbon from the hosts is split into around 100,000 seeds. The trisaccharide planteose in the dry seeds of root parasitic weeds is hydrolyzed rapidly after strigolactone perception, then sucrose and subsequently glucose are released to promote germination. In this study, we examined whether the planteose metabolic pathway could be a target for control of a broad range of root parasitic weeds, since planteose might be contained universally in Orobanchaceae seeds.

Because planteose is an α -galactosyl-sucrose, β -galactosidases in germinating seeds of *Orobanche minor* were characterized. Crude enzymes extracted from the germinating seeds showed significant β -galactosidase activity at pH 5, while weak activity was detected at pH 7. The β -galactosidase activity at pH 5 increased with the progress of germination up to seven days after GR24 treatment (DAG). When 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside was applied to the germinating seeds, β -galactosidase activity was detected near micropyles as blue spots at 5 DAG.

Three genes possibly encoding β -galactosidases were identified by transcriptome analysis on germinating seeds of *O. minor* and named OmAGAL1 - OmAGAL3. FPKM values showed that the expression level of OmAGAL2 significantly increased from 1 to 2 DAG. OmAGAL2 without a transit peptide at its N-terminus (Δ TP-OmAGAL2) expressed heterologously in *E. coli* exhibited β -galactosidase activity with an optimum at pH 5. Finally, it was confirmed that planteose was hydrolyzed by Δ TP-OmAGAL2 and released sucrose at pH 5. To investigate its localization, the full-length OmAGAL2 coding sequence (CDS) was fused with CDS of mCherry red fluorescent protein, and transiently expressed in the leaves of *Nicotiana benthamiana*. The red fluorescence image indicated that OmAGAL2:mCherry localized around the cells. Taken together, OmAGAL2 might hydrolyze planteose at acidic environment around the cells, i.e. apoplasts.

Screening of a chemical library composed of 150,000 compounds identified 28 OmAGAL2 inhibitors (PIs). When each PI was applied to the seeds of *O. minor* with GR24, significant suppression of radicle elongation was observed. Some PIs non-selectively suppressed the growth of *Arabidopsis*, while the other PIs exhibited less effects on *Arabidopsis* comparing with on *O. minor*.

In summary, OmAGAL2 was identified and characterized as the acid β -galactosidase capable of hydrolyzing planteose. The gene expression and localization profiles of OmAGAL2 suggested that planteose was hydrolyzed at apoplasts during the early germination process of *O. minor*. Since some PIs selectively suppressed the radicle elongation of *O. minor*, the planteose metabolic pathway, especially the OmAGAL2 orthologue, could be a target for control of a broad range of root parasitic weeds.

Wakabayashi, T., Joseph, B., Yasumoto, S., Akashi, T., Aoki, T., Harada, K., Muranaka, S., Bamba, T., Fukusaki, E., Takeuchi, Y., Yoneyama, K., Muranaka, T., Sugimoto, Y. and A. Okazawa, 2015. Planteose as a storage carbohydrate required for early stage of germination of *Orobanche minor* and its metabolism as a possible target for selective control. *J. Exp. Bot.* 66: 3085-3097



Tuesday 2 July 14.15 – 18.10 Molecules and Biochemistry

Cannalactone: a new non-canonical strigolactone exuded by *Cannabis sativa* roots with a pivotal role in host specialization within French broomrape (*Phelipanche ramosa*) populations.

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In France, two populations of the branched broomrape (*Phelipanche ramosa* L. Pomel) differ by their major host: genotype 1 preferably parasitizing rapeseed (*Brassica napus*) and genotype 2a parasitizing hemp (fiber strains of *Cannabis sativa*) (Stojanova et al., 2019). In comparison to genotype 1 seeds, genotype 2a seeds display a 10-fold less sensitivity to canonical strigolactones (SLs) (e.g., (\pm)-GR24, Boyer et al., 2014) but a 10 to 100-fold higher sensibility to hemp root exudates. By implementing a combined, parallel bio-guided purification strategy using hemp root exudates, a new compound correlating with differential activities on *P. ramosa* seeds and host preference was isolated. Indeed, this compound, which we named cannalactone, induces germination of genotype 2a seeds at much lower concentrations (nanomolar level) than genotype 1 seeds. Its molecular structure was determined by NMR and MS analyses and its absolute configuration is currently under investigation: cannalactone is a new non-canonical SL with three cycles (A, C and D). Low phosphate fertilization promotes cannalactone exudation from hemp roots. Other demonstrated biological activities of cannalactone include shoot branching inhibition in pea via the PsD14/RMS3 receptor and mycorrhizal symbiosis promotion between alfalfa and *Rhizophagus irregularis*. Biochemical analyses revealed its interaction-related hydrolysis by the SL receptors (PsD14/RMS3, AtD14) and another homologous α/β -hydrolase (AtKAI2). A biosynthetic pathway hypothesis of cannalactone from methyl carlactonoate, a key intermediate for the biosynthesis of non-canonical SLs, will be presented. These results confirm the wide structural diversity of SLs and demonstrate the structure specificity role in the host recognition of root parasitic plants.

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Boyer, F.-D., A. de Saint Germain, J.-B. Pouvreau, G. Clav  , J.-P. Pillot, A. Roux, A. Rasmussen, S. Depuydt, D. Lauressergues, N. Frei dit Frey, T.S.A. Heugebaert, C.V. Stevens, D. Geelen, S. Goormachtig and C. Rameau, 2014. New Strigolactone Analogs as Plant Hormones with Low Activities in the Rhizosphere. *Molecular Plant*. 7, 4: 675-690.



Wednesday 3 July 9.00 – 12.45 Control and management

Striga research on finger millet: Protocols, GWAS and RNA sequencing

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Finger millet (*Eleusine coracana* subsp. *coracana*; $2n=4x=36$) is the third most important cereal crop in the semi-arid regions of the world. The grain has an impressive nutritional profile, excellent storage qualities, and can grow under diverse agro-ecologies, including degraded farmland. One of the most important biotic stress in finger millet in the tropics is the parasitic weed, *Striga hermonthica*. Finger millet has only become a host to *S. hermonthica* over the last few years and the mechanism of response to *Striga* remains unknown. Traditionally, farmers have managed *Striga* in cereal fields through intercropping (Aasha et al. 2017) and crop rotations (Oswald and Ransom 2001) with edible legumes such as common bean (*Phaseolus vulgaris* L.), pigeonpea (*Cajanus cajan* (L.) Millsp.) and mung bean (*Vigna radiata* (L.) R. Wilczek). Studies on *Striga*-finger millet interactions are limited, if not non-existent. To enable a better understanding of finger millet in response to *Striga* attack, we optimised in vitro and glasshouse *Striga* screening conditions and validated our results using natural field screening conditions. To identify genomic regions likely associated with *Striga* resistance, we undertook a genome-wide association study (GWAS) using a panel of diverse finger millet breeding lines from Kenya. Phenotypic data was collected under natural conditions in two sites, two seasons. Genotypic data was generated using both genotyping-by-sequencing and DaRT-sequencing. The GWAS study was validated using RNA-sequencing of resistant lines versus susceptible ones during critical stages of *Striga* infestation. Our results demonstrate the power of “omics” tools in the efficient breeding of finger millet against parasitic weeds. Future studies will need to focus on better characterization of *Striga* (Li and Timko 2009). Wild relatives of finger millet will be valuable and should be screened alongside the landraces and cultivated accessions while looking for novel sources of resistance to *Striga*.

Aasha, M.B., H.E. Amani, M.A.A. Rashida, A.K. Nahid, 2017. Effects of intercropping pearl millet with some legumes on *Striga Hermonthica* emergence. SSRG Intl J Agri Environ Sci 4(6): 65-72

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Wednesday 3 July 9.00 – 12.45 Control and management

Parasitic weed management- opportunities and challenges

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The obligate root parasitic weeds commonly named broomrape (*Orobanche* and *Phelipanche* spp.) cause severe damage to vegetable and field crops worldwide. The arid and semi-arid conditions in the Mediterranean basin require the use of irrigation system to ensure optimal water supply in the dry spring and summer. Global warming is changing irrigation practices in areas where rains are scarce and flooding irrigation is not possible, thus requiring the use of more sophisticated irrigation systems (e.g. southern Europe, California). We hypothesized that integration of a modeling approach and chemical applications (foliar or delivered by drip irrigation- 'Herbigation') at the soil sub-surface broomrape development phase (pre broomrape attachment and/or post broomrape attachment), will control the parasite and prevent crop damage. In this presentation three protocols for broomrape management based on a decision support system (DSS) and precision herbicide applications at a farm scale, based on a modeling approach will be given: control of *P. aegyptiaca* in processing tomato, *O. cumana* in confectionery sunflower, and *P. aegyptiaca* in carrot. The use of the DSS lead to effective control of Egyptian broomrape in processing tomatoes commercial fields. In high infestation levels tomato yields increased by 40 tons per ha compared to the non-treated control. Eight large scale experiments that examined Herbigation of imazapic to control *O. cumana* in sunflower resulted in sunflower seed yield increase of 1.6 tons per ha (~40%). Twenty-five large-scale experiments examined foliar application of low glyphosate rates for *P. aegyptiaca* control in carrot confirmed 50% yield increase in heavily infested fields. As our modeling approach addresses only temporal variation of the parasitism dynamics, we have started to develop models to estimate the spatial variation using remote sensing under the concept of site-specific weed management (precision agriculture). In our current presentation, we will explore the state of the art image-driven models for early broomrape detection that are based on hyperspectral and 3-D host morphological analysis. Additionally, we show how application of 3-D segmentation methodology allowed estimation of organ-level parameters which were more affected by the broomrape infection. Advantages and disadvantages of 3-D host morphological analysis and hyperspectral-based detection approach will be compared.

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Eizenberg, H. and Y. Goldwasser, 2018. Feature Article: Control of Egyptian broomrape in processing tomato: a summary of 20 years of research and successful implementation. *Plant Disease* 102: 1477-1488



Wednesday 3 July 9.00 – 12.45 Control and management

Advances in parasitic weed control in the field

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It is known for many years that parasitic weeds of the genera *Striga* and *Orobancha* spp cause severe yield losses of important food crops, especially in developing countries and the Mediterranean. Although many attempts were made to control these weed pests, none of them is fully satisfactory. Due to the intensification of agriculture for food production, the problem becomes more and more serious. The use of common herbicides is not really effective, because food crops are damaged as well. Controlled use of Glyphosate is rather successful, but this agent is under heavy societal debate. The intriguing option is employing strigolactones as suicidal germination agent, where germination of the seeds of the parasite takes place in the absence of a host plant. Due to lack of nutrients, the germinated seeds will die. This method has been suggested for the first time in 1976. However, this strategy was not considered feasible in practice due to alleged untimely decomposition of the germinating agent. In spite of this doubtful image, we investigated several synthetic germination analogs for the control of parasitic weeds. We highlight the requirements for a successful application protocol: * Design a model for SL analogs, *selection of suitable strigolactone analogs, *optimal treatment of the infested soil, *time frame of the application, *precondition of seeds, *prevention of untimely decomposition of the selected strigolactone analogs, *avoidance of accumulation of stimulant in the soil, *environmental issues. Details of successful protocols will be presented. An alternative method involves the decomposition of the natural germination stimulant prior to its action. This implies that the stimulant will not reach the seeds. This goal can be achieved by treatment of the soil with a basic substance or a strong nucleophilic agent. * We will discuss the selection of a suitable base, * the choice of appropriate nucleophiles, *environmental and toxic aspects, * pros and cons and * successful protocols of this strategy.

The current status of parasitic weed control in the field will be critically reviewed.

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Zwanenburg, B. and D. Blanco-Ania, 2018. Strigolactones in the spotlight. *J Exp. Bot.* 69: 2205-2218



Wednesday 3 July 9.00 – 12.45 Control and management

Correctly multi-targeted Host-Induced Gene Silencing (HIGS) should allow full and sustainable control of parasitic weeds

Jonathan Gressel

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Transgenic plants were engineered 30 years ago to produce double stranded RNA molecules that entered pests, replicated themselves using the pest's machinery and spread throughout the pest. Double stranded dsRNA is processed in the pest into short 21-24 nucleotide long pieces that interact with pest mRNA suppressing its function. This "interference RNA" (RNAi) causes Host-Induced Gene Silencing (HIGS). RNAi can move both from host to parasite and parasite to host. *Phelipanche aegyptiaca*, as well as *Cuscuta pentagona* were statistically significantly suppressed by dsRNA emanating from hosts but insufficiently to be considered agronomically significant. HIGS research went on hold with parasitic weeds and other pests due to the low level of control. Interest in HIGS resurged with pathogens as >99% gene suppression was achieved. The new tactic: instead of using a single construct, simultaneous engineering of many different dsRNA producing constructs targeting many different sites on a gene and/or many different genes. A second issue may be a better choice of genes to target. There should also be a resurgence of interest in the technology among parasitic weed researchers – and they should consider targeting parasite genes that are differentially expressed soon after parasite attachment. Regulation of HIGS transformed crops should be easier than with crops transformed with structural genes as no proteins are produced. Resistance to multiple HIGS via gene mutations should take an exceedingly long time, as the simultaneous mutations at multiple sites would be at infinitesimally low frequencies, unlike single gene bred resistance. The only regulatory issue should be that the RNAi should not target any mammalian gene – easily ascertainable via database search. Because the RNAi producing constructs are small, many different ones can be "stacked" into a crop, along with dsRNA's targeting insects, nematodes, viruses, and pathogenic fungi. They need not be in a single large construct if introduced by biolistic transformation, where the majority of constructs enter the same site. Alternatively they can be added to a CRISPR-Cas9 gene editing disruption site. Engineering a package of stacked crop protection HIGS into the same site has the advantage that the whole package is inherited as a single dominant gene, easing the backcrossing into multiple elite varieties and inbreds of the target crop. Thus, multiple HIGS may provide an in seedo solution to many crop protection problems, when done based on knowledge not available a decade ago.

Gressel, J., 2018. Hit parasitic weeds hard with HIGS: they possibly can be transgenically controlled. *Haustorium* 73: 4-7 for references



Wednesday 3 July 9.00 – 12.45 Control and management

Realizing the suicidal germination strategy to control *Striga hermonthica* in rain-fed agriculture of sub-Saharan Africa

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The root parasitic plant *Striga hermonthica* is a major threat to global food security, causing enormous losses in yields of the main staple crops in sub-Saharan Africa, which include pearl millet, sorghum, maize and rice. Sustainable *Striga* control requires the depletion of the vast, long-lived *Striga* seedbank. Inducing lethal seed germination in hostTMs absence through application of strigolactone (SL) analogs is a promising approach that can significantly reduce or even eliminate accumulated *Striga* seedbanks. However, this suicidal germination strategy has been in general rarely tested and never evaluated under the natural rain-fed conditions that prevail in *Striga*-prone regions. In this study, we have developed and validated a protocol for suicidal germination in laboratory and natural conditions in *Striga*-infested rain-fed African fields. For this purpose, we assessed the effectiveness of three SL analogs, methyl phenlactonoate 1 (MP1), MP3 and Nijmegen-1 in lab, greenhouse and real rain-fed conditions, and developed a protocol for on-farm application. The established fit-for-purpose protocol proved to be very effective in inducing suicidal germination, resulting in up to 65% and 55% decrease in *Striga* emergence in pearl millet and sorghum fields, respectively. Our results demonstrate that suicidal germination is a successful method to reduce *Striga* seedbank, even in rain-fed agriculture. Moreover, the minimal demands of our protocol, in terms of water consumption and amount of selected SL analogs, make it affordable and applicable at a large scale in rain-fed agriculture, holding promise for sustainable cleaning of heavily *Striga*-infested fields in sub-Saharan Africa.

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Wednesday 3 July 9.00 – 12.45 Control and management

How do fertilizers affect the facultative parasitic weed *Rhamphicarpa fistulosa*?

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Rhamphicarpa fistulosa is a facultative parasitic plant, adapted to tropical wetlands (Hansen, 1975; Rodenburg et al., 2015). The species has developed into an invasive weed problem in rainfed lowland rice production systems in at least 28 countries in Africa, causing an estimated annual loss of 204,000 tonnes of paddy, worth \$82M (Rodenburg et al., 2016b). The weed is typically a problem on resource-poor smallholder farms, and reportedly associated with poor soils (Ouedraogo et al., 1999; N'Cho et al., 2014). Farmers' main curative control measure is hand weeding (N'Cho et al., 2014; Tippe et al., 2017a). We recently confirmed effectiveness of two preventive control options, i.e. resistant varieties (Rodenburg et al., 2016a) and early sowing (Tippe et al., 2017b). Because of the putative relation between poor soil fertility and *R. fistulosa* infestation, a third preventive control option we investigated was fertiliser application. Fertilisers have previously shown to suppress the obligate parasitic weed *Striga hermonthica* (e.g. Jamil et al., 2012). In a greenhouse pot-experiment in Wageningen (the Netherlands) and three consecutive years of field experimentation in Kyela (Tanzania), *R. fistulosa* growth with and without a host (rice) was assessed under fertilised and unfertilised growing conditions. In the pot experiment, the fertiliser treatment encompassed different levels of Di-ammonium phosphate (DAP). In the field, the fertiliser treatment consisted of the recommended dose of DAP (+ urea), NPK or organic soil amendments (rice husks or cattle manure) with or without half the recommended dose of DAP (+urea). All experiments included a no-fertiliser control. In pots, in absence of rice, biomass of *R. fistulosa* did not differ between fertiliser levels at the first sampling date (74 days after sowing), whereas at 121 days, it only increased following intermediate fertiliser application rates. In contrast, biomass of *R. fistulosa* growing with rice increased significantly with every increase in the fertiliser application rate at both dates (74 and 121 days). Such a stimulatory rather than a suppressive effect of fertilisers on the growth of *R. fistulosa* was also observed in the field. Once again, this effect was generally stronger in the presence of host plants. Differences among fertilisers varied between years but fertilisers with organic components generally resulted in the highest *R. fistulosa* biomass. In presence of a host crop, fertilisers have stimulatory effects on facultative parasitic weeds. In *R. fistulosa* infested fields, fertilisation should therefore only be promoted in combination with effective curative and preventive control measures.

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Wednesday 3 July 9.00 – 12.45 Control and management

Development of chickpea (*Cicer arietinum* L.) mutant resistant to imidazolinone herbicides for broomrape management

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Chickpea (*Cicer arietinum* L.) is an important crop in the crop rotation management in Israel. This crop suffers from both crenate (*Orobanche crenata*) and Egyptian broomrape (*Phelipanche aegyptiaca*). Imidazolinones (IMI), a group of acetolactate synthase (ALS) inhibitors, are effective for broomrape control (Eizenberg et al., 2013); however, chickpea plants are highly sensitive to this group of herbicides. To achieve imidazolinone resistance in chickpea, we produced chickpea ethyl methane sulfonate (EMS) mutagenized population based on an elite erect chickpea cultivar resistant to Fusarium wilt and Ascochyta blight, with high yield. In this study, we describe the identification of a novel mutation in the ALS1 gene of line 2033 found in the mutagenized population that confers herbicide resistance. This mutation results in an amino acid substitution located in a highly conserved region of the ALS protein that is involved in the binding site of IMI herbicides. Homozygous plants were found to be cross-resistant to all imidazolinone herbicides, but not to other chemical groups of ALS inhibitors. Tobacco plants were transformed with the mutated or with the wild type chickpea ALS1 gene. The tobacco plants harboring the mutated ALS1 gene were completely resistant, while the plants containing the normal ALS gene were sensitive to IMI herbicides as was the wild type. These results confirm the role of the mutation in IMI resistance. Field resistance of 2033 mutant was demonstrated in field experiments.

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Will not be presented due to illness: *Phelipanche ramosa* infestations and control in processing tomato in Chile

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An outbreak of severe infestations by the parasitic plant *Phelipanche ramosa* in processing tomato has been detected in Chile in recent years. The rapid spread and intensification of infestations has led the industry to define the control of this devastating parasite as a top priority in processing tomato management. To combat these infestations a mutual project was initiated in 2017 by the private non-profit foundation UC Davis Chile Life Sciences Innovation Center together with Israeli scientists, along with the financial and active support of the processing tomato company 'Sugal Chile'. In the 2017-2018 season we identified both morphologically and by specific molecular markers the broomrape species found in Chilean processing fields and defined the species as *Phelipanche ramosa* L. (Pomel). Initial experiments using the available Chilean sulfonyl-urea herbicides rimsulfuron, iodosulfuron, and halosulfuron, and the imidazolinone herbicide imazamox reduced *P. ramosa* infestation but did not provide adequate control. In the 2018-2019 season we conducted two field trials in two tomato growing regions- one in Curico and one in Penciahue. Broomrape control treatments were advised according to a tomato/broomrape phenological growing-degree-days decision support system, obtained from extensive laboratory, field studies research, and commercial management practices in Israel. Two main treatments were practiced: a. Pre-plant incorporated 37.5 g/ha Sulfosulfuron followed by 3 post applications of 4.7, 4.7 and 7.1 g/ha imazapic and applied via the drip irrigation system and a final 7.1 g/ha foliar imazapic application; b. Three Post-planting foliar applications of 37.5 g/ha sulfosulfuron and a final 7.1 g/ha foliar imazapic application. All sulfosulfuron treatments were activated by either drip or mini-sprinkler irrigation. In the Penciahue experiment all treatments drastically and significantly reduced broomrape parasitism with no adverse effect on tomato yields. The best treatment was the sprinkler activated b treatment which reduced *P. ramosa* inflorescence stem number and dry weight by 47 and 83 fold respectively, producing 99.5 ton/ha red tomatoes. There was no *P. ramosa* parasitism in the Curico field, thus the experiment served as a tomato phytotoxicity test. There was no yield reduction in all treatments compared to the non-treated control, confirming the safety of these treatments to processing tomato. Further studies will include field trials in experimental and semi-commercial plots, fine tuning of the herbicide applications regime based on the growing-degree-days decision support system, exploring the crop host range of the Chilean *P. ramosa* populations, and implementation of mapping, sanitation, and knowledge sharing methods.

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Thursday 4 July 9.00 – 12.45 Parasitic plant biology

Understanding the Arms Race: Host resistance and parasite virulence in the *Striga hermonthica*-rice interaction

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Striga species are obligate hemi-parasitic weeds that parasitise the roots of rain-fed rice, maize, sorghum and millet in sub-Saharan Africa (SSA), causing yield losses that range from ~30 to total crop failure. Effective control of *Striga* is essential for food security and poverty alleviation for subsistence farmers, but it remains elusive. The use of *Striga*-resistant varieties should form the cornerstone of integrated control programs, as resistance is recognized as sustainable and cost effective. However, their use is constrained by our limited knowledge of the identity, genetic basis and mode of action of genes underlying resistance to different *Striga* populations (accessions) and by the potential for rapid evolution of virulence in the parasite.

I will describe the identification of a highly significant *Striga*-resistance Quantitative Trait Locus (QTL) in two different mapping populations of rice, where the *Striga*-resistant parents are an *Oryza sativa* ssp. japonica variety (Nipponbare) or an *O. sativa* ssp. indica variety (IR64). Both varieties provide broad-spectrum resistance against different genetic accessions of *Striga hermonthica*. The QTL region is rich in genes encoding transposable elements, small peptides and a cluster of disease resistance proteins. I will present the evidence that the candidate resistance genes contribute to the resistance phenotype, discuss their possible mode of action and consider the types of *S. hermonthica* effector's / pathogenicity determinants that may either elicit a resistance response or allow some *S. hermonthica* individuals to overcome resistance in the host.



Thursday 4 July 9.00 – 12.45 Parasitic plant biology

Native parasitic plants: a solution of plant invasions worldwide?

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Plant invasions cause loss of biodiversity and degradation of habitats in ecosystems worldwide. The main concerns of traditional invasion biology were exclusively alien invaders but expansions of native species (native invaders) have recently been shown to have comparable effects on biota. Preventing further invasion, reduction of the invasive species and restoration of the original diversity represent a major global challenge.

Recent research has highlighted an emerging role for native parasitic plants as biological control suppressing invasive plants, thus aiding in restoration of infested habitats. Parasitic plants have traditionally been viewed as pests in agriculture and forestry but some of them have been shown to enhance biodiversity through their effects on plant communities and ecosystem processes such as nutrient cycling. Plant parasitism may specifically interfere with key processes such as symbiotic nitrogen fixation or clonal propagation by rhizomes that allow invaders to grow quickly or attain dominance. In the case of alien invasions, lack of coevolution may help explain why highly competitive invasive species are much more negatively affected by native parasitic plants than native hosts.

The most comprehensive evidence on parasitic plants interfering with plant invasions comes from three case studies testing the use of parasitic plants to suppress both alien and native invasive plants in Central Europe, southern Australia and eastern China. These studies demonstrate suppression of target invasive species, and in some cases an increase of native species abundance indicating recovery of the communities from invasions. Further cases of native parasitic plants attacking invasive species have been recorded across five continents.

Parasitic plants represent a ubiquitous component of terrestrial ecosystems worldwide. Native parasitic species are therefore available without the need to introduce additional alien species, which is the main concern of traditional biological control. They also display a variety of life forms and many species tend to be host generalists, enabling them to target invasive species of various growth forms and phylogenetic origins. To exploit the biological control potential of parasitic plants, further research should aim at: 1. Identifying compatible interactions between parasitic and invasive plant species, testing of these interactions and their collateral effects in infested natural communities, 2. Examining the characteristically high intraspecific genetic diversity of many parasitic plants and genotypic effects on parasite-host interactions, 3. Assessing possible risks, across all components of the ecological system, associated with transplanting and promoting the abundance of native parasitic plants in invaded habitats.

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Thursday 4 July 9.00 – 12.45 Parasitic plant biology

Transcriptomics to farmer field: a system biology approach for commercializing root parasitic *Santalum album* (Sandalwood)

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Sandalwood (*Santalum album* L.) is world famous for its scented heartwood and multi purpose essential oils. Sandalwood is an aggressive root hemiparasite and invading a suitable host accelerates its growth. Further, propagation through seeds create a huge genetic variation that reflects in quality and quantity of harvest. Because of these biological limitations, commercial scale cultivation of this high value crop is not popular. Present work focused on a system biology approach for addressing above major issues in commercial scale cultivations. Therefore, the objectives of the study were, to identify superior genotypes through gene specific marker assisted selection combined with biochemical composition, to propagate such genotypes through micropropagation and to identify a better host with an economical value while promoting the growth of Sandalwood at early stage. Row transcriptomics data in the public domain was used for assembling a transcriptome, identification of oil biosynthetic genes and developing SSR markers for important genes. The SSRs developed using newly developed bioinformatics pipeline was validated with laboratory data. Tissue specific expression patterns were studied and HPLC analysis confirmed association of gene expression and biochemical composition. SSR genotyping and HPLC analysis were used for selecting superior genotypes. A direct organogenesis protocol was optimized from axillary bud culture for commercial-scale clonal propagation of identified superior genotypes. Young Sandalwood plants were grown in a rhizotone system with different hosts with commercial value for identification of preferred hosts for early growth. Legumes are better hosts in general while Sandalwood, Butterfly pea (*Clitoria ternatea*) association resulted highest number of haustoria and best growth performance of Sandalwood. Butterfly pea flower is used in beverage industry and most parts of the plants are used for medicinal purposes. Therefore it will provide an additional income to the grower during early stage. Training and extension work continue to introduce our newly develop package to farmers and commercial growers.

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Thursday 4 July 9.00 – 12.45 Parasitic plant biology

Reproductive biology and pollination of *Cynomorium songaricum* (Cynomoriaceae)

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Cynomorium songaricum, which belongs to the only genus of family Cynomoriaceae, is a holoparasitic plant that parasitizes mainly the root of *Nitraria* in the desert. It is a monocarpic species with a life cycle of 2-5 years. *C. songaricum* is a commonly used herb in traditional Chinese medicine, which has led to a sharp decrease in the number of natural populations. The information on the reproductive biology of *C. songaricum* is scarce, which seriously restricts the development of artificial breeding and population conservation. Therefore, from 2016 to 2018, we systematically studied the flower development, floral biology, pollinators, breeding systems, temperature, and identification of volatiles in the inflorescences in the wild populations of *C. songaricum* in Alxa, Inner Mongolia, China. We found that the male flowers begin to develop after their emergence from the soil, the male and female flowers are mature at the same time. The numbers of inflorescences, male flowers, female flowers, and bisexual flowers per square centimeter were 62-132, 315-460, and 0-5, respectively. The total number of pollen grains per anther was 8,612-11,786. The mature female flowers showed stigmatic receptivity of 100%, reducing to 74.52% in senescent flowers. In mature anthers, 97.95% of pollen grains are viable, decreasing to 35.86% after 3 d. The male and female flowers of the inflorescence mature gradually, and the flowering duration of individual was 20-25 d. *C. songaricum* is a cross-pollination and self-incompatible plant with pollinators of Diptera (Anthomyiidae, Muscidae, Sarcophagidae, Calliphoridae, Tachinidae, and Eurystomatidae). Wind played a relatively minor role in pollination. The volatiles in the inflorescence of *C. songaricum* included benzaldehyde, butanoic acid, benzyl alcohol, nonanal, etc., which may have the activity of attracting carrion flies in carrion-mimicking flowers. The purplish red or dark brown inflorescences significantly increased the inflorescences temperature, promoting the emission of inflorescences volatiles, thus increasing the visiting rate of carrion or omnivorous flies.



Thursday 4 July 9.00 – 12.45 Parasitic plant biology

Triphysaria controls vegetative self-recognition by restricting release of HIFs in roots

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Plants secrete and respond to a wide array of chemicals to communicate in rhizosphere (Massalha et al. 2017). Root parasitic plants develop haustoria in response to haustoria inducing factors (HIFs) released by neighboring host plants (Yoshida et al. 2016). The ability to distinguish self from non-self in root parasitic plants presumably maximizes their beneficial connections with hosts. *Triphysaria* is a hemiparasite in Orobanchaceae and has a broad host range, while it rarely parasitizes other *Triphysaria* (Yoder 1997). We hypothesized that the chemical differences between host and parasite root exudates might contribute to the vegetative self-recognition phenomenon in *Triphysaria*. Bioassays of root exudates from three *Triphysaria* species and two host species (*Arabidopsis* and *Medicago*) showed that *Triphysaria* seedlings only formed haustoria with exudates from host roots but not parasite roots. One known HIF DMBQ was eluted in the most active fraction of *Arabidopsis* root exudate in HPLC. Quantification of DMBQ concentration in each root exudate via LC-QQQ-MS/MS revealed that the two host plants secreted similar levels of DMBQ while parasite root exudates contained no or very little amount of DMBQ. These results suggested that the difference in HIF concentration between host and parasite root exudates may account for the vegetative self-recognition in *Triphysaria*. Previous studies reported DMBQ could be produced from phenolic compounds by phenol oxidases. By exogenously applying commercial phenol oxidases (horseradish peroxidase and fungal laccase) to intact *Arabidopsis* and *Triphysaria* roots, we found that functional HIFs were released from both host and parasite roots, but DMBQ was released only from *Arabidopsis* roots. We have generated multiple transgenic *Triphysaria* lines to constitutively overexpress *Arabidopsis* peroxidase and fungal laccase genes *in vivo* and we are currently phenotyping these transgenic roots to find out if self-recognition in *Triphysaria* could be partially lost. Our results suggested that HIFs could be produced from *Triphysaria* roots by phenol oxidases and that *Triphysaria* might restrict the spacial or temporal expression of proper phenol oxidases, such as peroxidases or laccases, in order to prevent self-parasitism. In the long term, our study of self-recognition in parasitic plants may be useful agriculturally by applying similar strategies into crop plants to make them invisible to parasitic weeds.

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Thursday 4 July 9.00 – 12.45 Parasitic plant biology

Analysis of genetic variation in pre and post attachment resistance mechanisms in maize inbred lines to the parasitic weed *Striga hermonthica*; implications for control

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Maize is one of the major staple, cereal crops in sub-Saharan Africa (SSA) but many maize varieties used by farmers are very susceptible to the obligate, root parasitic weed, *Striga hermonthica*. *S. hermonthica* seeds are widespread, genetically variable and long-lived in soils of cereal growing regions and the parasite represents one of the major biotic threats limiting maize production in SSA. Control of this parasite is essential to improve crop yields and food security for farmers and the use of resistant varieties should play an important role in integrated management practices, but maize varieties with durable resistance are lacking. Plant breeders at IITA, Ibadan Nigeria, have developed maize inbred lines with excellent sources of resistance derived from a wild relative of maize (*Zea diploperennis*), African landraces and other tropical germplasm. Intensive screening of the inbred lines in the field under artificial *S. hermonthica* infestation has identified a range of inbred lines with high levels of resistance to *S. hermonthica*. However, screening germplasm for resistance to *S. hermonthica* in the field does not provide an insight into the basic mechanisms of resistance, or reveal whether resistance is broad-spectrum against different accessions of the parasite. Thus understanding the resistance mechanisms and their mode of action in different inbred lines is an essential pre-requisite for efficient exploitation of host plant resistance as a *Striga* control measure.

In this study we quantified post-attachment resistance in 25 maize inbred lines to two different accessions of *S. hermonthica* collected from two locations (Abuja and Mokwa) in Nigeria and characterized the phenotype of resistance in the roots. In addition, we quantified the amounts and types of strigolactone germination stimulants in the maize root exudates and analysed their biological activity on parasite seed germination, as a measure of pre-attachment resistance. The maize inbred lines exhibited a broad range of susceptibility/resistance reactions to the different *S. hermonthica* accessions. Most of the maize lines showed very good levels of post attachment resistance to both *S. hermonthica* accessions from Abuja and Mokwa but some of the lines were susceptible to *S. hermonthica* from Abuja illustrating the importance of understanding the molecular genetic basis of host-parasite specificity. The lines also exhibited considerable differences in the amounts and types of strigolactones produced in maize root exudates, varying from high to low producers, imparting possible differences in pre-attachment resistance. These results are discussed in the context of breeding resistance to *S. hermonthica*.



Thursday 4 July 9.00 – 12.45 Parasitic plant biology

Impact of the soil microbiome on Striga-sorghum interaction

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The soil microbiome is known to contribute to plant defense against pathogens and insects pests (Raaijmakers & Mazzola, 2016; Pineda et al., 2017). However, its impact on the interaction between parasitic weeds and their host is poorly understood. To assess the role that the microbiome plays in the interaction, we screened 24 physicochemically and microbiologically diverse soil samples, collected from agricultural fields across the Netherlands, for parasitic weed suppressiveness. The result showed substantial variation in Striga suppression between plants grown in the different soils, ranging from 12 to 73 Striga attachments per plant. To investigate if the soil microbiome influences Striga attachment to Sorghum roots, untreated and gamma-irradiated portions of the most suppressive soil were used in a Striga infestation assay. When two susceptible Sorghum accessions were grown in gamma-sterilized soils, the number of Striga attachments per unit of root dry weight significantly increased when compared to plants grown in untreated soils. These results indicated that the soil microbiome affects Striga incidence in Sorghum. Similarly, an assay involving four resistant accessions indicated that gamma-sterilization of the soil significantly increased Striga attachment per unit of root dry weight in two out of the four resistant accessions. Collectively, our results highlight the importance of the soil microbiome in the interaction between the parasitic weed Striga and its host plant Sorghum and that Striga suppression operates in a soil-plant genotype dependent manner. Currently we are investigating the members of the soil microbiome and the microbial functions that confer Striga-suppression.

Pineda A, Kaplan I, Bezemer TM. 2017. Steering Soil Microbiomes to Suppress Aboveground Insect Pests. Trends Plant Sci 22(9): 770-778.

Raaijmakers JM, Mazzola M. 2016. Soil immune responses. Science 352(6292): 1392-1393.



Thursday 4 July 14.15 – 18.00 Parasitic plant-host interaction

War and Peace – The Molecular Dynamics of Compatible and Incompatible Striga -Host Plant Associations

Michael P. Timko, Chun Su, Hai Liu, Eric K. Wafula, Loren Honaas, Claude W. de Pamphilis,

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Cowpea (*Vigna unguiculata* L.) is the most important food and forage legume in the African Sahel providing essential protein nutrition and income to millions of farmers. While most cowpea cultivars are susceptible to the root parasitic weed *Striga gesnerioides*, cultivar B301 is resistant to all known parasite races except SG4z. When challenged by races SG4 and SG3, the roots of B301 display a hypersensitive response (HR) at the site of parasite attachment followed by death of the invading parasite. In contrast, no visible response occurs in B301 roots parasitized by SG4z and the parasite successfully penetrates the host root cortex, forms vascular connections, and grows to maturity. Comparative transcriptomics and in silico computational analysis uncovered a set of secreted host effectors capable of altering various aspects of host innate immunity. Among these are small leucine-rich receptor (LRR)-protein kinase (PK) homologs that specifically target components of the host resistance response signal transduction cascade and lead to a suppression of HR elicitation. The mechanism of parasite suppression of host innate immunity will be discussed as well as how these new findings could potentially contribute to the development of novel strategies for controlling *Striga* and other parasitic weeds thereby enhancing crop productivity and food security globally.



Thursday 4 July 14.15 – 18.00 Parasitic plant-host interaction

What model plants can tell us about parasitic plants

Thomas Spallek

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The Orobanchaceae is a plant family with more than 2,000 root-parasitic plant species, including agricultural relevant species of the genera *Striga*, *Orobanche*, *Phelipanche* and *Rhizophicarpa*. Parasitism evolved likely only once within this family, suggesting that key aspects of parasitism are shared. However, the genes and molecular processes driving parasitism remain largely undiscovered. Aiming to identify these genes, I present our recent work on the model parasite *Phtheirospermum japonicum*. Based on our previous work on *Phtheirospermum*-mediated cytokinin transfer to host plants during parasite-induced host hypertrophy, I developed a procedure to screen a *P. japonicum* EMS-mutagenized M2 population for mutants that fail to parasitize *Arabidopsis thaliana* plants despite successful infection. The ongoing screening and re-screening have so far resulted in the isolation of two *P. japonicum* \do not alter on host plants\ (dalton) mutants. I will introduce these mutants and discuss why identifying the underlying dalton mutations may have implications beyond the studied model system.

T. Spallek, et al., 2017. Interspecies hormonal control of host root morphology by parasitic plants. *Proc. Natl. Acad. Sci. U.S.A.* 114(20):5283-5288.



Thursday 4 July 14.15 – 18.00 Parasitic plant-host interaction

Message received: Evidence for translation of mobile mRNAs in *Cuscuta*-host interactions

So-Yon Park, Vivian Bernal-Galeano and James H. Westwood

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Messenger RNAs (mRNAs) readily move between *Cuscuta* and its hosts, forming a remarkable example of interspecies sharing of nucleic acids (Kim et al. 2014). This exchange is potentially a mechanism for the parasite or host to influence growth of - or gain information from - the interacting plant, but the function of mobile mRNAs remains unclear in the parasite-host association. One possible fate of mobile mRNAs is translation into protein after reaching the recipient organism, with the resulting protein potentially affecting the destination cell. We have taken a multi-pronged approach to exploring the possibility of mobile mRNA translation, including use of reporter genes encoded by mobile mRNAs, TRAP (Translating Ribosome Affinity Purification; Zanetti et al., 2005), and proteomics. First, we studied *Arabidopsis* plants that express versions of the GUS reporter gene with or without a mRNA mobility-conferring motif (Zhang et al., 2016), and found that the host-to-*Cuscuta* movement of GUS mRNA was correlated with the appearance of blue in the parasite stems, indicating GUS enzyme activity. We verified that the GUS protein itself did not appear to be mobile in this system, but additional evidence is needed. A second approach to evaluating translation was conducted using TRAP, in which epitope tagged *Arabidopsis* ribosomes facilitated the purification of ribosomal complexes and the presence of specific bound *Cuscuta* mRNAs. Finally, we assayed protein content of *Arabidopsis* and *Cuscuta* growing in association with each other, and found that hundreds of host and parasite proteins are present in the corresponding non-self organism. Based on the large size and insolubility of some proteins, as well as results from ¹⁵N labeling experiments, we conclude that some proteins were likely synthesized in the destination organism. Taken together, our results point to a complex *Cuscuta*-host interaction in which proteins from one species are found in another as a result of either direct protein mobility or translation of mobile mRNAs. The function of mobile proteins in this system requires further research.

Kim, G., M.L. LeBlanc, E.K. Wafula, C.W. dePamphilis and J.H. Westwood. 2014. Genomic-scale exchange of mRNA between a parasitic plant and its hosts. *Science* 345: 808-811

Zhang W.N., C.J. Thieme, G. Kollwig, F. Apelt, L. Yang, N. Winter, N. Andresen, D. Walther and F. Kragler. 2016. tRNA-related sequences trigger systemic mRNA transport in plants. *Plant Cell* 28: 1237-1249

Zanetti M.E., I.-F. Chang, F. Gong, D.W. Galbraith and J. Bailey-Serres. 2005. Immunopurification of polyribosomal complexes of *Arabidopsis* for global analysis of gene expression. *Plant Physiol.* 138: 624-635



Thursday 4 July 14.15 – 18.00 Parasitic plant-host interaction

The roles and functions of lignin in parasitic plant-host interaction

Songkui Cui, J. Musembi Mutuku, Syogo Wada, Yuri Takeda, Toshiaki Umezawa, Ken Shirasu, Yuki Tobimatsu, Satoko Yoshida

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Lignin is a complex aromatic polymer ubiquitously present in the secondary cell wall of vascular plants. Lignin-related monomeric compounds act as important host signals, namely haustorium inducing factors (HIFs), to induce haustorium in many Orobanchaceae members, yet their biological significance in plant parasitism remains unclear. To address this question, we investigated the roles of host lignin for haustorium induction in parasitic plants, and resistance against the obligate parasitic plant, *Striga hermonthica*. Structural specificity of lignin polymers and related monomeric compounds exists for haustorium induction in *Striga* and the facultative parasite *Phtheirospermum japonicum*. Manipulation of the host lignin composition affects both haustorium inducing capacity and post-attachment resistance. To gain insights into how HIFs are perceived by parasitic plants, we also investigated cellular accumulation of HIFs during haustorial formation using fluorescence-tagged monolignols, which appeared to be able to induce haustoria in *Striga*. Our results show that, upon haustorium induction, monolignols are immediately incorporated into the cell wall of haustorial epidermis, indicating that they are polymerized into lignin at haustorial surface. Such lignification may assist further infection processes by providing haustorium additional mechanical strength and/or physical barrier. Our studies demonstrate that lignin is an important player in host-parasitic plant interaction, particularly at early stage of infection that involves formation and functional establishment of haustorium.

Yoshida, S., Cui, S., Ichihashi, Y., and Shirasu, K. (2016). The haustorium, a specialized invasive organ in parasitic plants. *Annu Rev Plant Biol* 67: 643-667.

Cui, S., Wada, S., Tobimatsu, Y., Takeda, Y., Saucet, S.B., Takano, T., Umezawa, T., Shirasu, K., and Yoshida, S. (2018). Host lignin composition affects haustorium induction in the parasitic plants *Phtheirospermum japonicum* and *Striga hermonthica*. *New Phytol* 218: 710-723.

Mutuku, J.M., Cui, S., Hori, C., Takeda, Y., Tobimatsu, Y., Nakabayashi, R., Mori, T., Saito, K., Demura, T., Umezawa, T., Yoshida, S., and Shirasu, K. (2019). The structural integrity of lignin is crucial for resistance against *Striga hermonthica* parasitism in rice. *Plant Physiol* 179: 1796-1809.



Thursday 4 July 14.15 – 18.00 Parasitic plant-host interaction

A receptor and pathways discovered in the lignin-based resistance to *Cuscuta campestris* in Heinz hybrid tomato cultivars

Min-Yao Jhu, Moran Farhi, Richard Philbrook, Li Wang, Hokuto Nakayama, Kristina Zumstein, Neelima Sinha

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Parasitic angiosperms directly attach to host plants using specialized organs known as haustoria, which function as physiological bridges to extract nutrients and water from their hosts. *Cuscuta* species (dodders) are common and agriculturally destructive flowering stem parasitic plants. Reports have shown a 50–72% reduction in tomato yield when attacked by dodders. The physiological connection between host plants and parasites makes traditional herbicides and control methods ineffective. The Heinz hybrid cultivars H9492 and H9553 exhibit resistance to dodders. The stem cortex in these lines responds with local lignification upon *C. campestris* attachment causing the *C. campestris* strand to fall off. To identify the key resistant genes, we focused on genes that have different expression patterns under *C. campestris* infestation in the resistant cultivars, compared to susceptible cultivars. Based on these criteria, we identified an AP2-like transcription factor, MYB55, and CC-NBS-LRR as key resistant genes. The transient overexpression of MYB55 and AP2-like induced stem lignification in the susceptible cultivar. These results suggest that MYB55 and AP2-like may directly regulate the biosynthesis of lignin in the cortex. Therefore, we termed this AP2-like protein as LRF1 (Lignin-based resistance factor 1). On the other hand, overexpression of this CC-NBS-LRR only induced lignification upon *C. campestris* attachments. This result indicates that this CC-NBS-LRR functions as a receptor for receiving *C. campestris* signals, thereby leading to the lignification-based resistance. Thus, we named it CuLiRR1 (*Cuscuta*-induced lignin-based resistance receptor). We also identified a transcription factor WRKY16 as a negative regulator of the lignin-based resistance. WRKY16 CRISPRed plants also induced lignification in the cortex and became more resistant to *C. campestris*. The results of this study provide the starting point for developing a parasitic plant-resistant system in crops.

Hegenauer V, Färst U, Kaiser B, et al., 2016. Detection of the plant parasite *Cuscuta reflexa* by a tomato cell surface receptor. *Science* 353, 478-81.

Hembree KJ, Lanini W, Va N. Tomato varieties show promise of dodder control. *Proceedings of the Proc. Calif. Weed Sci. Soc*, 1999, 205-6.

Kaiser B, Vogg G, Färst UB, Albert M, 2015. Parasitic plants of the genus *Cuscuta* and their interaction with susceptible and resistant host plants. *Frontiers in Plant Science* 6, 45.



Thursday 4 July 14.15 – 18.00 Parasitic plant-host interaction

Cytokinins act as signaling molecules within the rhizosphere to trigger haustorium formation in the holoparasitic plant *Phelipanche ramosa*

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The heterotrophic lifestyle of parasitic plants relies on the development of a haustorium, a key infectious organ required for attachment and invasion of the host root, connection to the vascular system and spoliation of host's resources. While haustorium development was shown to be initiated upon chemodetection of host derived phenolic compounds in either facultative (*Triphysaria versicolor*, *Phtheirospermum japonicum*) and obligate hemiparasitic plants (*Striga* sp), the induction of haustorium formation remains largely misunderstood in holoparasitic species such as *Phelipanche ramosa* and none of the already identified Haustorium Inducing Factor (HIF) are efficient to induce this development process in *P. ramosa*.

We showed that the root exudates of the host plant *Brassica napus* contain allelochemicals displaying a haustorium inducing activity on *P. ramosa* germinating seeds which increases the parasite aggressiveness towards host plants. A transcriptomic approach during early haustorium formation upon treatment with *B. napus* root exudates allowed the identification of differentially expressed genes involved in hormone signaling. Interestingly, bio-guided HPLC fractionation of various host plants (*B. napus*, *Arabidopsis thaliana* and *Solanum lycopersicum*) root exudates showed that haustorium inducing activity was constantly present in the same two fractions (nr 9 and 16). Bioassays using exogenous cytokinins and the specific cytokinin receptor inhibitor PI55 showed that cytokinins induced haustorium formation and increased parasite aggressiveness. Moreover, the overexpression of cytokinin marker genes in *P. ramosa* germinating seeds was shown in response to cytokinins, crude root exudates and fraction 9 but not fraction 16. Bio-guided UPLC-MS analysis showed that fraction 9 of *B. napus* root exudates contain a cytokinin with dihydrozeatin characteristics. In order to confirm the host derived origin of cytokinins detected in root exudates and if so to better understand the exudation pathway, we used *A. thaliana* mutant plants for cytokinin biosynthesis, transports or degradation. Exudates from mutants showed a variation in the induced haustorial activity, with a decrease for biosynthesis mutants. These results suggest that cytokinins, constitutively exuded from host roots, play a major role in haustorium formation and aggressiveness in *P. ramosa* and emphasize the role of cytokinins as signal molecules in the rhizosphere. In addition, our findings showed that host plants exudates also contain yet un-characterized non-cytokinin HIFs (fraction 16), thus presenting the cue governing haustorium induction as a more complex signal.

Goyet V., Billard E., Pouvreau J.B., Lechat M.M., Pelletier S., Bahut M., Monteau F., Špichal L., Delavault, P., Montiel G., and Simier P., (2017). Haustorium initiation in the obligate parasitic plant *Phelipanche ramosa* involves a host exudated cytokinin signal. *Journal of Experimental Botany* 68(20):5539-5552.



Thursday 4 July 14.15 – 18.00 Parasitic plant-host interaction

Striking vegetative developmental convergence in endoparasitic angiosperms

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A defining feature of all flowering plant parasites is their haustorium, which is a unique feeding organ that establishes connection with their host's vasculature. Among the 12 independent origins of plant parasitism, most species grow largely exterior to their host. The exception is a subset of holoparasites, which grow almost entirely within their host species and emerge only briefly during reproduction. Until recently, these so-called endoparasites were thought to represent a single clade united by their shared reduced vegetative morphology. We now know that these species represent four distinct evolutionary origins of this extreme form of parasitism, thus offering a new opportunity to examine convergence of this life history. Here, for the first time to our knowledge, we present a comprehensive examination of the morphology, anatomy and development of six holoparasitic species representing the four major endophytic plant clades: Apodanthaceae, Cytinaceae, Mitrastemonaceae and Rafflesiaceae. Our results demonstrate that colonization of the host xylem follows a common developmental trajectory. All endophytes establish initially as parenchymatic cells and differentiate to form conductive xylem cells, which establish direct connections to their host's vessels. Alteration of host xylogenesis i.e. hypertrophy and hyperplasia of host xylem cells was documented in all host-parasite interactions and is a common feature of plant parasitism. Apodanthaceae and Rafflesiaceae species additionally differentiate phloem tissue to form sieve tube elements that connect directly with sieve tube elements of their hosts. Considering the established effects of increased auxin concentrations in the vascular cambium, as well as cases of reverse haustorium flux (from parasite to host), our observations support the hypothesis that parasitic plants manipulate host cambium activity, likely by altering auxin concentrations during development. An additional unique anatomy was observed in the host vines of Rafflesiaceae, *Tetrastigma*, in which host sieve tube elements of the phloem were observed to differentiate towards parasitic tissues. This is distinctive among endoparasitic angiosperms and raises the hypothesis that Rafflesiaceae influence both host xylem and phloem differentiation, suggesting a wider form of parasitic plant action over host cambium activity. Finally, by mapping endophytic system proliferation within the host body using microtomography techniques, we discuss the hypothesis of infestation by multiple parasitic individuals on a single host root/stem.



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[P1] Structural and biochemical characterization of strigolactone parasitic receptors, understanding their functionality and how to inhibit them.

Amir A. Arellano Saab, Peter Stogios, Alexei Savchenko, Peter McCourt.

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Savchenko Laboratory, Biozone Centre for Applied Bioscience and Bioengineering, University of Toronto. 200 College Street, Toronto, ON, M5S3E5 Canada.

Striga hermonthica is the most destructive food crop parasite in the developing world. It invades its hosts by perceiving a group of host-derived small molecules called strigolactones. The receptors in charge of sensing strigolactones are encoded by a group of a/b hydrolases that not only recognize strigolactones but also hydrolyze them. The mechanisms of how SL receptors perceive their ligands and the role of hydrolysis in this perception are still rudimentary explained. To this end, I have crystallized three a/b hydrolases, two from parasitic plants in complex with inhibitory compounds (ShHTL7 and ShHTL9) and one from *Arabidopsis* (DLK2). Using these structures, I carried out molecular dynamics (MD) simulations, which led us to hypothesize about the role of hydrolysis and conformational change in protein signalling. At the same time, I have used the crystallography information obtained to determine the cause for hypersensitivity of some strigolactone receptors, creating hybrid proteins with specific hydrolytic abilities. Ultimately, the data generated will aid in the development of strigolactone agonists and antagonists that perturb the germination of the parasite *Striga hermonthica*.

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[P2]Deciphering the sorghum root microbiome for Striga-suppressive bacteria

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Striga is one of the most devastating parasitic weeds in Sub-Saharan Africa causing substantial yield losses of multiple crops including sorghum. Several strategies have been pursued to control Striga, ranging from cultural practices to breeding for resistance. However, the Striga problem remained at large to date with severe socio-economic impacts. The main obstacle limiting the effectiveness weed control measures is most of the damage is done while Striga is still underground. Therefore, disrupting the life-cycle of this parasitic weed at the soil-plant interface is crucial. This work aimed at identifying root-associated microorganisms that can interfere with the initial stages of root infection. To this end, we investigated the taxonomic and functional diversity of the root-associated microbiome of 12 sorghum accessions, including wild relatives, landraces and Striga-tolerant sorghum accessions. Three microbiologically and physicochemically distinct Ethiopian soils collected from sorghum-growing fields were used. Metataxonomic analyses of the sorghum rhizosphere microbiome by 16S-rRNA amplicon sequencing revealed that 94% of the total phyla is comprised of Proteobacteria (33%), Actinobacteria (30%), Acidobacteria (18%) and Firmicutes (13%). Based on a multivariate analysis we showed that both soil type and sorghum genotype significantly affect the sorghum rhizosphere microbiome assembly. Comparative analysis of the rhizosphere microbiome of Striga-susceptible and Striga-tolerant sorghum accessions showed distinct compositional variation in a soil type dependent manner. The core microbiome of Striga susceptible and tolerant sorghum genotypes is dominated by members of the Actinobacteria and Proteobacteria. Analysis of the functional traits of these Actinobacteria and Proteobacteria and their impact on Striga infection of sorghum roots is subject of ongoing and future experiments. Strategies will be presented on how to use this knowledge to engineer a Striga-protective sorghum microbiome.

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[P3]Genome-wide association analysis of Striga resistance in early maturing tropical maize inbred lines

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Striga hermonthica is a devastating parasitic weed causing significant reduction in maize yield in sub-Saharan Africa. Breeding for improved resistance to *Striga hermonthica* among tropical maize germplasm could be greatly enhanced by identifying SNPs associated with Striga adaptive traits. This study was conducted to determine the population structure and identify candidate markers through genome wide association mapping (GWAS) of 132 early maturing maize inbred lines with resistance to *Striga hermonthica* parasitism. These maize lines were phenotyped under artificial Striga infestation at Mokwa, a Striga endemic location in Nigeria, for two years and further sequenced with 7,224 DArT-seq markers. The measured traits showed moderate to high heritability estimates and significant correlations were observed between the Striga resistance indicator traits and grain yield. The inbred lines were grouped into two major genetic clusters by the STRUCTURE software. The GWAS analysis identified SNPs significantly associated ($P < 0.001$) with key Striga resistance indicator traits: 7 SNPs were significantly associated with Striga damage at 8 weeks after planting (8WAP), 5 with Striga damage at 10WAP, 7 with ears per plant and 3 with grain yield. Preliminary results showed that allelic variations at each significant SNP was associated with 22 to 32% of the phenotypic variance. After validation, the significant loci identified in this study could be targets for breeders in marker-assisted selection to accelerate genetic enhancement of maize for Striga resistance in the tropics, particularly in the West and Central Africa sub-region.

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[P4] Adaptation of sorghum landraces across gradients of *Striga hermonthica* occurrence

Emily S. Bellis, Victoria L. Deleo, Elizabeth A. Kelly, Claire M. Lorts, Germinal Rouhan, Andrew P. Budden, Govinal Badiger Bhaskara, Zhenbin Hu, Robert Muscarella, Thomas E. Juenger, Michael P. Timko, Geoffrey P. Morris, Claude W. dePamphilis, Jesse R. Lasky

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Host-parasite coevolution can maintain high levels of diversity in traits involved in species interactions. To leverage patterns of genome diversity for identifying genes involved in host resistance, we developed a framework to extend genotype-environment association analyses to the case of biotic environmental gradients. We constructed species distribution models for the parasitic weed *Striga hermonthica* and used genomic data from over 2,000 sorghum landraces to identify regions of the Sorghum bicolor genome strongly associated with *S. hermonthica* distribution. We found diverse loss-of-function alleles in the known sorghum resistance locus LGS1 exhibit statistical associations with predicted distribution of *S. hermonthica* and are geographically widespread among sorghum landraces, supporting a role in local adaptation to parasitic plant occurrence. However, low frequency of these alleles outside of *S. hermonthica* endemic areas suggests tradeoffs could limit their distribution. In support of tradeoffs related to endogenous function of strigolactones in hosts, we observed elevated expression of strigolactone biosynthesis pathway genes in root transcriptomes of resistant vs. susceptible sorghum varieties under nutrient stress. Genome-wide analyses revealed additional loci with evidence for locally adaptive natural variation including genes involved in cell wall modification, lignin deposition, and strigolactone perception. An excess of high-frequency polymorphism surrounding candidate loci supports a role for balancing selection in the long-term maintenance of allelic diversity contributing to host resistance in the sorghum-*Striga hermonthica* pathosystem.



[P5] *Cuscuta campestris*: Transformation and other tools for parasitic plant research

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Parasitic plants live by taking their nutritional needs from other plants through specialized structures called haustoria. *Cuscuta* species (dodders) are weedy stem parasites and are totally dependent on their hosts to complete their life cycle (holoparasites). Recent studies have revealed new biological phenomena involved in host-*Cuscuta* interactions like molecular recognition of the parasite (Hegenauer et al, 2016) by the host, and bidirectional exchange of mobile mRNAs (Kim et al, 2014), microRNAs (Shahid et al, 2018) and proteins (Birschwilks et al, 2007) through the haustorium. Interest in *Cuscuta* research is increasing, but researchers new to *Cuscuta* can be frustrated by challenges of working with this organism. No standardized protocols exist for *Cuscuta* research, nor has a protocol for stable genetic transformation of *Cuscuta* been developed. We are developing methodologies for studying parasite-host interactions in order to promote consistency in research and provide tools for functional genetic studies. We have developed standardized methodologies for growing *Cuscuta campestris* under lab conditions considering host, light quality, propagule type, etc. For the goal of stable transformation of *C. campestris*, we have tested different types of tissues for callus production and transformation, including seedlings, shoots, meristems, flowers, and immature embryos. We have obtained callus-like tissues under tissue culture conditions, regenerated shoots, and successfully transferred these cultured shoots to hosts. We have tested several transformation methodologies, both Agro-bacterium-dependent and independent, and have obtained transient transformation of *Cuscuta* tissues, but we have not yet succeeded in regenerating shoots from transformed sectors. Additional work is ongoing to overcome this barrier. Together, these methodologies will promote *Cuscuta* research and facilitate characterization of genes that are important for *Cuscuta* parasitism.

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[P6]Hybrid-Type Strigolactone Analogues and Mimics Derived from Auxins

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Strigolactones (SLs) constitute a new class of plant hormones of increasing importance in plant science. Unfortunately, the scarce availability of SLs and their structural complexity limit their applicability for the benefit of humankind and renders the synthesis of analogs and mimics with the same activity as the only solution to overcome this problem.

We present herewith new hybrid-type SL analogs and mimics derived from auxins, synthesized via coupling of two auxin esters [ethyl 2-phenylacetate and ethyl 2-(1H-indol-3-yl)acetate] with four D-rings (mono-, two di- and trimethylated). The new hybrid-type SL analogs and mimics were bioassayed for germination activity of seeds of the parasitic weeds *S. hermonthica*, *O. minor* and *P. ramosa* using the classical method of counting germinated seeds and a colorimetric method. The bioassays revealed that the analogs and mimics with a natural monomethylated D-ring had an appreciable to good activity towards the three species and were the most active derivatives. The new hybrid-type analogs may be attractive as potential suicidal germination agents for parasitic weed control.

Adéla Hýlová, Tomáš Pospíšil, Lukáš Spíchal, Jurgen J. Mateman, Daniel Blanco-Ania and Binne Zwanenburg, 2019. New Hybrid Type Strigolactone Mimics Derived from Plant Growth Regulator Auxin. *New Biotechnology* 48: 76–82



[P7]Unfitting pipes! Patterns of connection between mistletoes and their hosts: anatomical and hydraulic consequences for angiosperms parasitizing conifers

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Mistletoes are hemiparasitic plants capable of connecting their vascular systems to those of their hosts. These connections enable parasites to extract sap with water, minerals and few organic compounds. Because plants of different phylogenetic origins may engage in these host-parasite connections, they must be able to integrate different anatomic organizations. This work aimed to understand how angiosperm parasites can connect and function when parasitizing conifers. In this work, we present some of these connections and discuss some of the major constraints. We employed several approaches, since traditional paraffin or methacrylate inclusion, conventional wood anatomy, conventional and confocal microscopy, and X-ray microtomography. For hydraulic architecture experiments, we used dye infusions in vivo, pressure pump, and porometer for stomata transpiration measurements. Because of the economic losses on timber production in the literature the most studied model is *Arceuthobium* (Santalaceae) parasitizing several conifers. This first case has parenchyma-like cells bearing or not protoplasts on the boundary mainly the sap conduction from a dead cell (tracheid) to a living cell (parasite endophyte) or connective parenchyma. Other connections can be very different, such as the medium-sized mistletoe *Phoradendron juniperinum* (Santalaceae) on *Juniperus occidentale* (Cupressaceae) from Arizona (US), the large mistletoe *Psittacanthus schiedeana* (Loranthaceae) on *Pinus* (Pinaceae) from Veracruz (Mexico), or the long and branched *Struthanthus flexicaulis* (Loranthaceae) that spreads all over the crown of *Podocarpus lambertii* (Podocarpaceae), both from Atlantic rain forest (Brazil). In these last three host-parasite interactions the parasites are larger than the tiny *Arceuthobium*, exhibiting higher transpiration demands, due to their large crowns, with many broad leaves and poor stomata control. *Phoradendron juniperinum* shows indirect vascular connections mediated by connective parenchyma. *Psittacanthus schiedeana* has a single primary haustorium, connecting its vessels to tracheids, with long sinkers. *Struthanthus flexicaulis* has several small holdfasts attached to all the crown of *Podocarpus* with uncountable connections between the two vascular systems. The last two cases show important modifications in the pits and rare vessel elements, sometimes as this as tracheids. What must be considered is that for the studied pairs the parasite must be able to absorb sap from a conifer wood, with water potentials much lower than the usual for angiosperms. Also, they need to deal with pits that bear torus that can cause blocking of the water flow and xylem failure. In spite of all these constraints, mistletoes are very successful parasitizing conifers often leading the host to death.

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[P8]Phenotyping of early stages of wild *Helianthus* species/ *Orobanche cumana* interaction towards the identification of new resistances

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Orobanche cumana (the sunflower broomrape) is an obligate parasitic plant that specifically infects sunflower and is one of the main constraint of sunflower crop in Europe. Genetic resistance is the most efficient control of this parasitic weed illustrated by the breeding for resistant varieties in the past decades. However, more virulent broomrape populations are frequently emerging in many countries by overcoming the resistances introgressed in the cultivated sunflower varieties. Building durable resistance requires the combination of various resistance mechanisms and origins, named pyramidal resistance (Velasco et al., 2016), in contrast to vertical monogenic resistance. Searching for new and complementary broomrape resistance sources and mechanisms is hence a priority for sunflower breeding. Wild *Helianthus* species have been shown to provide such resources (Seiler and Jan, 2014). With this purpose, a screen of wild *Helianthus* species has been undertaken, through phenotyping of various stages of their interaction with the most virulent races of *O. cumana*: broomrape seed germination and haustorium formation using *Helianthus* root exudates, early stages (attachment and tubercle development) in mini-rhizotrons, and emerged shoots in pots. For early stages, a phenotyping tool in mini-rhizotrons (Louarn et al., 2016) has been optimised through the set-up of the nutritive solution, image acquisition using a nano-computer Raspberry/ cameraPi, and image analysis using Image J software. Sixty accessions (wild *Helianthus* and wild *H. annuus*) including *H. grosseserratus*, *H. tuberosus*, *H. anomalus*, *H. divaricatus*, *H. bolanderi*, *H. argophyllus*, *H. petiolaris*, *H. praecox*, *H. debilis*, *H. nuttallii*, *H. strumosus* and *H. pauciflorus* are being screened in the project. First results will be presented including cytological studies.

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[P9]Breeding strategies for *Orobanche cumana* resistance in sunflower

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Broomrape (*Orobanche cumana* Wallr.) is one of the most important constraints on sunflower production in Europe and Asia. It produces a large number of small seeds that are easily disseminated, leading to the build-up of *O. cumana* populations, and the constant appearance of new, and more virulent races. Current racial situation of broomrape in the main infested areas is unclear, since there is a lack of information on whether races under the same name reported in different countries are the same or differ in terms of virulence. Among the several control options proposed, breeding for resistance have been found to be most effective and environmental friendly way. Since *O. cumana* resistance is broken frequently, multiple sources of resistance are needed to control the emerging races. In a line with this goal, the Institute of Field and Vegetable Crops Novi Sad has been testing the wild relatives of the sunflower (long-term program) as well as inbred lines in gene bank (short-term program) and identified sources that confer resistance to highly virulent races. Some resistance sources have been found to be controlled by major genes, some have recessive inheritance, but some showed QTL resistance. Race-specific dominant genes are considered as good sources of resistance, but emerging of new races increase the aggressiveness and breakdown vertical resistance. Therefore, 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. The complexity of host resistance has been also improved using molecular marker analyses for identifying and mapping resistance genes.

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[P10] Duplicative horizontal gene transfer of mitochondrial *atp8* gene observed in *Cistanche armena* (Orobanchaceae)

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Parasitic plants establish a close physical connection with their hosts and such an association of their tissues seems to facilitate horizontal gene transfers (HGT), which is especially likely to affect their mitochondria. The obligatory parasites from the family richest in holoparasitic species, Orobanchaceae, form a haustorium at the early stage of development with which they establish a life long association with their host. This makes those plants a natural target for HGT focused research. Studies of mitochondrial genes in chosen species of holoparasitic Orobanchaceae were performed to search for occurrences of HGT. One of the obtained sequences, the *atp8* gene of *Cistanche armena* (K. Koch) M.V. Agab., displayed an additive polymorphism. Belonging to the Orobanchaceae family, *C. armena* is a rare, critically endangered, and poorly known parasitic plant that is endemic to Armenia and occurs only in Ararat Province. This obligatory parasite has a very narrow host range, limited to only two species belonging to different families: *Salsola dendroides* (Chenopodiaceae) and *Alhagi maurorum* (Fabaceae). The obtained sequence of its mitochondrial gene that displayed an additive polymorphism might indicate the presence of multiple copies of the gene. To confirm that, molecular cloning was performed and it proved the presence of two versions of the *atp8* gene in the genome of *C. armena*. As expected, one of the copies (later described as native) was similar to those found in other members of the Orobanchaceae family, while the other (later described as foreign) differed from them. Further research showed the similarity of this foreign copy of the *atp8* gene to those observed in the plants belonging to a distant plant family, Chenopodiaceae, especially to the genus *Salsola*, to which belongs one of its hosts-*S. dendroides*. This similarity indicates that, most probably, it was horizontally transferred to *C. armena* from its host. When comparing the transferred gene with the native *atp8* copy, only nucleotide substitutions were found and no evidence of open reading frame shifts mutations. The detected transfer is an example of duplicative HGT, where the foreign gene is present in the genome in addition to its native copy.



[P11]Development of chickpea (*Cicer arietinum* L.) mutant resistant to imidazolinone herbicides for broomrape management

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Chickpea (*Cicer arietinum* L.) is an important crop in the crop rotation management in Israel. This crop suffers from both crenate (*Orobanche crenata*) and Egyptian broomrape (*Phelipanche aegyptiaca*). Imidazolinones (IMI), a group of acetolactate synthase (ALS) inhibitors, are effective for broomrape control (Eizenberg et al., 2013); however, chickpea plants are highly sensitive to this group of herbicides. To achieve imidazolinone resistance in chickpea, we produced chickpea ethyl methane sulfonate (EMS) mutagenized population based on an elite erect chickpea cultivar resistant to Fusarium wilt and Ascochyta blight, with high yield. In this study, we describe the identification of a novel mutation in the ALS1 gene of line 2033 found in the mutagenized population that confers herbicide resistance. This mutation results in an amino acid substitution located in a highly conserved region of the ALS protein that is involved in the binding site of IMI herbicides. Homozygous plants were found to be cross-resistant to all imidazolinone herbicides, but not to other chemical groups of ALS inhibitors. Tobacco plants were transformed with the mutated or with the wild type chickpea ALS1 gene. The tobacco plants harboring the mutated ALS1 gene were completely resistant, while the plants containing the normal ALS gene were sensitive to IMI herbicides as was the wild type. These results confirm the role of the mutation in IMI resistance. Field resistance of 2033 mutant was demonstrated in field experiments.

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[P12]How do shifts in parasitism affect chloroplast genomes in the Santalales?

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Compared to their autotrophic relatives, parasitic plants often have plastid genomes (plastomes) that are smaller, have lost several genes, and have higher substitution rates in the genes that are retained. Almost half of all known species of parasitic plants belong to the order Santalales. As the order contains the full range of parasitic lifestyles, from non-parasitic species to hemiparasites and holoparasites, it is an ideal candidate clade for studies of plastome evolution in relation to parasitism. To date there have been no comprehensive comparative studies of plastome evolution across the Santalales including both non-parasites and holoparasites. Our aim is to investigate patterns of plastome evolution in the Santalales from a phylogenetic perspective. To augment existing published plastomes, we aim to assemble approximately 70 new plastomes, targeting both autotrophic and holoparasitic representatives in addition to hemiparasites. At the genome level, we will investigate patterns of rearrangements, reductions of non-coding sequences, and changes to the borders of the inverted repeats. We will also investigate patterns of gene evolution, including substitution rate changes, pseudogenization and gene loss, and we will test if evidence for loss of purifying selection can be observed on individual genes.

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[P13]Metabolic and Biochemical Aspects of Interaction between Species of Carrots to Root Parasite

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Carrots (*Daucus carota*) are an important agricultural crop supplied to consumers throughout the year. It's used in food, pharmaceutical and cosmetic industries- due to its health benefits and the biological availability of the active components, like carotenoids, dietary fiber and minerals, its consumption is growing. Farmers are currently struggling with major pests affecting the growth and crop levels. For example, Orobanche and Phelipanche.

Phelipanche Aegyptiaca is a chlorophyll lacking root parasite that parasitizes many plants and causes direct damage to carrot crops. As a result, the yield and the quality of the vegetables are reduced. The interaction between the parasite (Phelipanche) and the host (carrot) includes various aspects of communication between two different plants, we are aware of some of them but we are not aware of others, because not all of them have been studied yet. These interactions include the transfer of water, nutrients and signals which are expressed in different metabolic pathways.

Carrots are considered to be a major growth field in Israeli crops, and they are known as a host to *P. aegyptiaca* and *O. crenata*. The carrot farmers aim to reduce the use of dangerous products for humans and the environment as a part of the global trend to use friendly means in modern agriculture. Understanding the mechanism of transferring macromolecules between carrots and *P. aegyptiaca* will prevent their interaction and directly reduce its damage. In our study, we investigate the genetic and metabolic relationship between *D. carota* and *P. aegyptiaca*, by characterizing and quantifying the carotenoids profile by high-performance liquid chromatography (HPLC) device in all root examples, and examining some genes' expression level that are involved in the biosynthesis pathway of carotenoids and strigolactones by RT-qPCR device.

First, we identified significant differences in size, shape, and carrot colour between *Phelipanche*-infested and non-infested carrots. This difference has caused a decrease in the amounts of pigments and the concentration of carotenoids produced by the plant compared to the control carrots. Furthermore, expression analysis of the investigated genes showed a significant reduction in their transcript levels in the *Phelipanche*-infested carrot. In addition, this research is the first to examine the concentration of carotenoids in the *Phelipanche* tubercle, which found small carotenoids concentrations. We concluded that the parasite affects the internal processes of the host plant for its own need, which might be by transferring carotenoids or through expressing several enzymes and factors that are present in the host plant to help itself.



[P14] Holoparasitism breaks the evolutionary stasis of mitochondrial genome evolution in Orobanchaceae

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The parasitic lifestyle in plants has brought about extreme genomic modifications, including dramatic functional and physical reductions to the plastid genome (plastome), accelerated rates of molecular evolution across apparently all genomic compartments, losses of essential mitochondrial genes for respiratory complex I (in mistletoes), and massive horizontal gene transfers. While the reductive evolution of plastomes is well understood as a consequence of relaxed selective constraints on photosynthesis, we still have little knowledge of the reorganizations pertaining to mitochondrial genomes (mitogenomes) in parasitic lineages. To infer the evolutionary trajectories and underlying causes of mitogenome reconfiguration in parasites, we completely sequenced and assembled the mitogenomes of 30 species from 20 genera covering all trophic transitions in the Broomrape family (Orobanchaceae). We find that mitogenome structure varies greatly, ranging in size from 316 kb to 3.58 Mb. Despite the enormous differences in their overall size, the length of homologous DNA remains conserved at 200–300 kb between the various species. Phylogenomic analyses indicate that mitogenome inflation is due to the gain of novel DNA and through incorporation of nuclear fragments. Also, the extent of apparently horizontally acquired genetic material shows a significant correlation with mitogenome size. In contrast, dispersed repeats and plastid-derived sequences contribute only marginally to mitogenome expansion. Holoparasites, especially those belonging to the broomrape clade, show a tendency towards larger mitogenomes, even though they have lost more protein genes than hemiparasites. Further, we observed that the reduction of plastid genomes is reversely proportional to mitogenome evolution, providing the first evidence for a linkage of the structural and molecular evolution between organellar genomes as parasitism unfolds..

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[P15]Element Distribution at *Cuscuta*/Host Infection Sites Suggests the Existence of Selective Mineral Transport Barriers

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Parasitic plants depend on a feeding connection with their hosts for supply of inorganic nutrients. Up to date there is very little known about the transfer of nutritional elements from the host plant to the parasite. Using X-ray fluorescence spectroscopy (XRF) (1), we analyzed the element composition of macro- and micronutrients, i.e. the “ionome”, at infection sites of the parasitic angiosperm *Cuscuta reflexa* growing on hosts of the genus *Pelargonium*. To determine whether the tissue type that is infected has an impact on the elemental distribution patterns within the infection sites, we compared cross sections through infection sites at main stems, petioles and pedicels of the host plants. Imaging methods combining XRF with 2-D (micro-XRF) or 3-D (confocal micro-XRF) microscopy show that most of the measured elements are present at the same or slightly higher concentrations in the parasite compared to the host in all infection sites. However, a few elements show sharply reduced levels (75-90% reduction) in *Cuscuta*. Calcium, strontium and manganese levels drop pronouncedly at the host/parasite interface, and manganese appears to accumulate in the host tissue surrounding the interface region. In contrast, chlorine is present in the haustoria at similar levels as in the host tissues but is sharply decreased in the stem of the parasite. Our observations thus indicate a restricted uptake of calcium, strontium, manganese and chlorine by the parasite. We thus conclude that inorganic nutrient uptake by the parasite *Cuscuta* is regulated by specific selective barriers whose existence has evaded detection until now.

The nature of this selectivity is not known so far. In photosynthetic plants nutrient transport and distribution is depending on the localization and activity of a plethora of specific membrane transporters and channels, but is also determined by the mobility of the nutrients in xylem and phloem (2). While all mineral nutrients can be transported in the xylem, several, but not all elements can be remobilized and redistributed by transport in the phloem. Interestingly, calcium and in most plant species also manganese are immobile in the phloem. Thus, the low calcium and manganese levels in *Cuscuta* might be explained by their immobility in the phloem, which also implies that the transport of nutrients in older, mature haustoria occurs mainly or solely through phloem but not xylem.

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[P16] Effects of environmental conditions on survival of a bird-dispersed mistletoe, *Phoradendron leucarpum*

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Mistletoes are a group of shrubs that typically parasitize the branches of host trees and rely on avian frugivores for seed dispersal. Because mistletoes are restricted to a narrow range of suitable recruitment sites (Mellado & Zamora 2014) and avian frugivores are more visible than other guilds of seed dispersers, mistletoe-frugivore systems afford opportunities for determining the roles of dispersal limitation and local environment in determining plant distribution (Martínez del Río et al. 1996, Carlo & Aukema 2005). We used a seed sowing experiment to quantify the roles of light availability and flood regime in determining the initial survival of oak mistletoe (*Phoradendron leucarpum*). During winters (Jan-Mar) 2016-2018 we planted 1000 oak mistletoe seeds across 50 plots in forested wetlands in southeastern Virginia on a variety of potential host tree species. In winter 2018 we planted 580 oak mistletoe seeds on potted red maple (*Acer rubrum*) saplings under a range of light availability and flood regime treatments using a split plot design. Mistletoe seedling survival and establishment data were analyzed using generalized linear models and the results suggest that light availability has a significant effect on seedling establishment. This information will be coupled with results from a regional co-occurrence study using presence-absence data on both oak mistletoe and avian frugivores to determine the relative influences of environmental conditions and seed disperser behavior on oak mistletoe distribution

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[P17] Identification of Differentially Expressed Genes Associated with Tolerance to *Striga hermonthica* in Tropical Maize Inbred Lines

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Striga hermonthica is a very important biotic constraint to maize production in Africa. Although maize lines with resistance to the parasite have been identified, the underlying mechanisms of this resistance have not been well characterized. To gain better understanding of the mechanism of maize response to *Striga* infestation, we have used RNAseq to profile the transcriptome of a susceptible (5057) and a tolerant (ZD05) maize genotype. Root tissue was collected from both susceptible (S) and resistant (R) plants either infested (I) or un-infested (U) at three time points corresponding to: attachment stage (3 dpi), intermediate stage (9 dpi) and late stage (22 dpi). Sequencing generated millions of paired-end reads which were processed and mapped to maize genome (Maize B73 RefGen_v4). Differentially expressed genes (DEG) were identified by analysing Cufflinks data by means of CummeRbund (Bioconductor) in RStudio. Differential expression was declared at log2 fold change ≤ -1.5 for downregulated and log2 fold change $\geq +1.5$ for upregulated genes at $p \leq 0.01$. Transcriptome analysis revealed that the resistance reaction was characterized by up-regulation of defence genes, cellular transporters, and genes involved in secondary metabolism. These include cell wall rearrangement genes including cellulose synthase, callose synthase, hydroxyproline-rich glycoprotein (HRGP), defence related as chitinase, phenylalanine ammonia lyase (PAL), chalcone synthase, chalcone flavone isomerase, stress responsive protein, trehalose-6-phosphate phosphatase and genes in the DIMBOA biosynthetic pathway. The susceptible reaction was characterized by up-regulation of some abiotic stress genes and down-regulation of a number of genes involved in secondary metabolism, defence and cellular transport. The susceptible genotype upregulated a number of heat shock protein genes as well as genes involved in response to reactive oxygen species, heat, drought and pathogens. These gene expressions in the resistant genotype at the third time point was similar to expression of the susceptible genotype at the first time point. This indicates that the resistant genotype delayed the effects of the parasite. These results suggest that ZD05 resist *Striga* infection using multiple biochemical and physiological mechanisms implying that the resistance is likely polygenic, as reported in other species. In addition, the resistant line mobilized a more comprehensive response to parasite infection by up-regulating more genes involved in plant secondary metabolism and defence. Two-pronged approach, including mini-rhizotron experiment and quantitative real-time PCR are underway to confirm the expression of candidate genes. Understanding the genes conditioning resistance to *S. hermonthica* will facilitate the development of functional markers for accelerating resistance breeding.



[P18] Structural basis for specific inhibition of the highly sensitive ShHTL7 receptor

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Striga hermonthica is a root parasitic plant that infests cereals, decimating yields, particularly in subSaharan Africa. For germination, *Striga* seeds require host released strigolactones that are perceived by the family of HYPOSENSITIVE to LIGHT (ShHTL) receptors. Inhibiting seed germination would thus be a promising approach for combating *Striga*. However, there are currently no strigolactone antagonists that specifically block ShHTLs and do not bind to DWARF14, the homologous strigolactone receptor of the host. Here, we show that the octyl phenol ethoxylate Triton X100 inhibits *S. hermonthica* seed germination without affecting host plants. High resolution X-ray structures reveal that Triton X100 specifically plugs the catalytic pocket of ShHTL7. ShHTL7 specific inhibition by Triton X100 demonstrates the dominant role of this particular ShHTL receptor for *Striga* germination. Our structural analysis provides a rationale for the broad specificity and high sensitivity of ShHTL7, and reveals that strigolactones trigger structural changes in ShHTL7 that are required for downstream signaling. Our findings identify Triton and the related 2[4(2,4,4 trimethylpentanyl)phenoxy]acetic acid as promising lead compounds for the rational design of efficient *Striga* specific herbicides.

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[P19]Uncovering How and When *Cuscuta campestris* Recognizes a Host to Produce Inter-species miRNAs.

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Cuscuta campestris is an obligate parasitic plant which attaches to host stems through a specialized organ called the haustoria to divert water and nutrients from the host to the parasite. Previous research has shown that inter-species microRNAs (miRNA) produced by *Cuscuta* have the capability of moving into the host and targeting mRNAs involved in multiple biological processes. While prehaustoria formation is characterized, it is unknown how *Cuscuta* is able to recognize a suitable host to begin mature haustoria formation and host penetration. For example, *Cuscuta* will not produce mature haustoria or miRNA when attached to bamboo stakes, but will when attached to *Arabidopsis thaliana* stems. Furthermore, the time frame of when miRNA production begins after host recognition and the specific cells responsible for their production have not been studied. In order to identify a “host recognition molecule”, cotton string impregnated with cytokinin, pectin, or sucrose in a variety of concentrations and combinations served as an artificial host for *Cuscuta* to attach. Pilot experiments suggest that pectin may play a partial role in host recognition. Interfaces between the parasite and string were collected and sectioned to check for mature haustoria formation. A time course experiment using *A. thaliana* or *S. lycopersicum* as a host was performed, harvesting *Cuscuta*/host stem interfaces every 24 hours after initial coiling for 14 days. Interfaces will either be subjected to in situ hybridization or sRNA sequencing to identify miRNA cellular localization and the time frame which miRNA production begins, respectively



[P20] Studies on *Thonningia sanguinea* Vahl. (Balanophoraceae) in Southern Nigeria: I. Range and Host Preference

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Studies on host-parasite relationship are intrinsically link with the nature of parasitic plants. No such records have been recorded for *Thonningia sanguinea* (Balanophoraceae), a rare, cryptic, obligate holoparasitic plant endemic to tropical Africa. This study reports on its host range and preference of in southern Nigeria. Its presence and current distribution ranges were plotted from reconnaissance surveys conducted across selected forested areas in southern Nigeria, comprising, national parks, forest reserves and community-managed forests. To detect specific hosts, soil excavation leading from parasite to host was conducted. The results showed a broad host range with special preference for native trees and members of the Euphorbiaceae and Urticaceae. Among the common host trees were: *Guarea cedrata* (Meliaceae), *Lophira alata* (Ochanaceae), *Musanga cecropiodes* (Urticaceae), *Myrianthus arboreus* (Urticaceae), and *Ricinodendron heudelotii* (Euphorbiaceae). Only *Hevea brasiliensis* and *Theobroma cacao* were of exotic origin. *Lophira alata* was the most susceptible host with a percentage infestation rate of 28.77, while *Musanga cecropiodes* had the highest percentage occurrence of plant as host with 31.57 %. The pattern of host range and preference of *T. sanguinea* could have significant implications on the conservation status of the plant, which accounting for current IUCN status is Not Evaluated (NE). In situ conservation is however recommended

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[P21] Studies on *Thonningia sanguinea* Vahl. (Balanophoraceae): II. Reproductive Phenology, Sex Ratio and Insect Pollinators at the Okomu National Park, Southern Nigeria

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A fundamental requirement for understanding the reproductive biology of cryptic species is to study its phenology, sex-ratio and identify potential pollinators. Phenological data comprising a frequency of flowering and fruiting and time sequence for each reproductive phenophase were collected through bi-monthly visits to sites harbouring the parasite at the Okomu National Park. The relationship between its phenology (flowering and fruiting) and rainfall was performed using the Spearman correlation analysis. To identify potential pollinators of *T. sanguinea*, the composition and rate of faunal visitors were recorded, collected, identified and data subjected to Berger-Parker index analysis. Phenologically, *T. sanguinea* flowers all-year-round. The frequency of flowering in male and female plants was relatively synchronized with peak values around June to November, while the Period of fruiting was observed to have peak values around October to February. A highly significant correlation was observed between the frequency of flowering and rainfall pattern ($R=0.917$; $P=0.000$). Overall, the incidence of female inflorescences surpassed male inflorescence by approximately 2:1 ratio. The pooled data on sex distribution among *T. sanguinea* sites reveals that 71% were inhabited by single-sex type, while the other 29 % showed co-occurrence of both sex types (potential monoecious specimens). The ant *Technomyrmex* species were the most common floral visitors, accounting for 55.54% frequency occurrence. The long reproductive phenology period allows for an all-year-round supply of food resources even in a season of scarcity. This could be one of the keystone roles it plays in forested habitat.

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[P22] Studies on *Thonningia sanguinea* Vahl. (Balanophoraceae) in Southern Nigeria: III. Distribution, Habitat Characteristics and Phytosociology

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Habitat characteristics and distribution ranges are important ecological information required for making conservation management decisions for rare plant species. *Thonningia sanguinea* Vahl (Balanophoraceae), apart from its recognition as root parasites of forest trees, information on the nature of its population and distribution range in Nigeria has not been reported. To delineate its presence and current distribution ranges, reconnaissance surveys across selected forested areas in southern Nigeria were used to delineate its presence and current distribution ranges. Records on habitat characteristics comprising host type, elevation, GPS location/Orientation, companion plant species, and soil types were collected from sampling plots from a 10m x 10m quadrats distributed in twenty-four (24) locations. The presence of *T. sanguinea* was observed in most forests types of southern Nigeria with ferrallitic and ferruginous soils, however no indication of its presence around the savanna-like region of the southeast. A total of 47 populations were recorded. Phytosociological studies indicate the close association with *Cercestis* sp, *Harugana madagascariensis*, *Anchomanes difformis*, *Musanga cecropiodes*, *Strombosia grandifolia*, *Icacina trichantia*, *Myrianthus arboreus*. Species diversity indices of sites reveal that except for species diversity ($P=0.02$), others such as species richness ($P=0.07$), number of individual species ($P=0.17$), and species evenness ($P=0.43$) of both conserved and disturbed sites were not significant. Its presence along footpaths, disturbed portion of the forest and preference for key secondary forest re-growth species such as *Musanga cecropiodes* and *Myrianthus arboreus* as host suggest it as a potential bio-indicator in predicting environmental degradation in a forested ecosystem.

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[P23] Studies on *Thonningia sanguinea* Vahl. (Balanophoraceae) In Southern Nigeria: IV. Patterns of Genetic Diversity and Population Structure within and between Populations

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No previous attempt has been carried to assess genetic variability in *Thonningia sanguinea* population in southern Nigeria, hence; the present study was conducted to elucidate the pattern of genetic variability and population structure among populations in southern Nigeria. We extracted genomic DNA from 31 individuals in 15 populations and tested them eight (8) random amplified polymorphic DNA primers. Several genetic diversity parameters were tested using GenALEX software Ver. 6.5. Reproducible RAPD markers indicate that all populations sampled are composed of individuals with high genetic variability. Populations were grouped into four distinct clades; with Populations from Okour community forest having high gene diversity and Shannon index. However, the population from Cross River National Park had the lowest Gene diversity and Shannon index value. Genetic variability did not correlate with geographic distances. Analysis of molecular variance revealed that most or (82.3%) of the diversity can be explained by allelic variations within the population. an Indirect estimate of gene flow gave an Nm value of 1.09, which indicates a low level of migration among populations. The results demonstrate that populations of *T. sanguinea* in southern Nigeria exhibit an out-crossing strategy expected of sexual exchange of gametes by different individuals within a local population

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[P24]Validation of broomrape resistance QTLs in sunflower line HA-267

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Broomrape (*Orobancha cumana*) is a parasitic weed that causes substantial yield losses in sunflower. While chemical options for suppressing sunflower broomrape are available, breeding for resistance has both economic and environmental advantages. In a previous study, we reported on the mapping of polygenic broomrape resistance in the HA-267xOD-DI-82 mapping population. We identified 2 major quantitative trait loci (QTL), or7.1 and or12.1, as well as numerous small effect QTLs that were dispersed across the genome. While these results provided valuable insights into the complexity of resistance to broomrape, QTL validation is an important and often overlooked step for breeding. The aim of this study is to assess the effects of these QTLs in an unrelated genetic background and to identify QTLs that are stable and thus have the highest breeding value. To achieve this goal, resistant line HA-267 was crossed with a susceptible parental line HA-26-PR, and a mapping population consisting of 189 individuals was developed. Following phenotyping, we used selective bulked segregant analyses coupled with genotyping-by-sequencing (GBS) to re-map QTLs conferring resistance to broomrape population LP12BSR. A total of 7 QTLs were identified. The results confirmed that the effects of QTLs or7.1 and or12.1 was large and consistent. Importantly, an additional QTL located on sunflower chromosome 3 (or3.1), which was previously proven to control resistance to broomrape race E, appeared to have a larger effect in the new HA-267xHA-26-PR cross compared to the previous one (HA-267xOD-DI-82). These validated and stable QTLs will be prioritized in breeding, and associated molecular markers will facilitate marker-assisted selection.



[P25] A new generation of methyl phenlactonoate strigolactone analogs with high efficiency and simple structure

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Strigolactones (SLs) are a group of carotenoid derived plant hormones that regulate plant growth and development as well as plant response to environmental stimuli. Besides this hormonal function, SLs serve as signaling molecules in plant's communication with mycorrhizal fungi, and are best known for their role in inducing seed germination of root parasitic plants. These biological activities predestine SLs for a wide range of agricultural applications. However, synthesis of natural SLs is laborious because of their complex structure and stereochemistry. To address this challenge, we have previously developed a series of simple SL analogs, named as "Methyl Phenlactonoates (MPs)", based on the structure of the SL carlactonoic acid. Here, we designed and tested a new series of easy-to-synthesize MPs. The new analogs were evaluated with respect to several SL activities, including regulation of plant architecture, inducing leaf senescence and induction of parasitic seed germination. These tests unraveled MP16 as the most efficient SL analogs among the new MPs. Moreover, some of the new MPs were more active in regulating *Arabidopsis* shoot and root architecture and in inducing seed germination in *Phelipanche aegyptica* than the widely used standard SL analog GR24. Taken together, the new MP series contains very promising candidates for various applications as SL analogs, which include large scale field application for combating *Striga* in infested regions of sub-Saharan Africa.



[P26] A receptor and pathways discovered in the lignin-based resistance to *Cuscuta campestris* in Heinz hybrid tomato cultivars

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Parasitic angiosperms directly attach to host plants using specialized organs known as haustoria, which function as physiological bridges to extract nutrients and water from their hosts. *Cuscuta* species (dodders) are common and agriculturally destructive flowering stem parasitic plants. Reports have shown a 50–72% reduction in tomato yield when attacked by dodders. The physiological connection between host plants and parasites makes traditional herbicides and control methods ineffective. The Heinz hybrid cultivars H9492 and H9553 exhibit resistance to dodders. The stem cortex in these lines responds with local lignification upon *C. campestris* attachment causing the *C. campestris* strand to fall off. To identify the key resistant genes, we focused on genes that have different expression patterns under *C. campestris* infestation in the resistant cultivars, compared to susceptible cultivars. Based on these criteria, we identified an AP2-like transcription factor, MYB55, and CC-NBS-LRR as key resistant genes. The transient overexpression of MYB55 and AP2-like induced stem lignification in the susceptible cultivar. These results suggest that MYB55 and AP2-like may directly regulate the biosynthesis of lignin in the cortex. Therefore, we termed this AP2-like protein as LRF1 (Lignin-based resistance factor 1). On the other hand, overexpression of this CC-NBS-LRR only induced lignification upon *C. campestris* attachments. This result indicates that this CC-NBS-LRR functions as a receptor for receiving *C. campestris* signals, thereby leading to the lignification-based resistance. Thus, we named it CuLiRR1 (*Cuscuta*-induced lignin-based resistance receptor). We also identified a transcription factor WRKY16 as a negative regulator of the lignin-based resistance. WRKY16 CRISPRed plants also induced lignification in the cortex and became more resistant to *C. campestris*. The results of this study provide the starting point for developing a parasitic plant-resistant system in crops.

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[P27] Sequence divergence among trans-species small RNAs in parasitic plant genus *Cuscuta* compensates for target-site diversity in hosts

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Cuscuta is a genus of vine-like parasitic plants that constrict and invade the stems of host plants, forming a symplastic interface through a specialized organ called a haustorium. We recently showed that *Cuscuta campestris* produces a cohort of miRNAs that enter the host and regulate expression of targeted host mRNAs (Shahid et al. 2018). The discovery of this form of parasitic effector led us to investigate if these miRNAs are produced by other closely related *Cuscuta* species. We performed sRNA sequencing on 7 different *Cuscuta* isolates from 4 species, sampling both the parasite-host haustorial interface and adjacent tissues from attachments to *A. thaliana*. Analysis revealed 300-800 small RNAs that are up-regulated in the interface of each isolate, most being sizes common to miRNAs (21 or 22 nucleotides in length). We identified 61 *A. thaliana* mRNAs that were targeted by induced *Cuscuta* small RNAs among the surveyed isolates, based on the presence of secondary siRNA accumulation and degradome analysis. Conservation of confirmed targets ranged from highly common (found in 3 out of 4 species) to extremely specific (only found in a single isolate from one species), with many target classifications including involvement in hormone signaling, plant-defense pathways, protein phosphorylation and the phloem. Surprisingly, almost no small RNAs were identical between species, with only a subset identical even within different isolates from a species. Clustering small RNAs into broader groups based on similarity revealed that many could be grouped into superfamilies that span across multiple species of *Cuscuta*. Looking closely target homologs among eudicots shows extreme amino acid conservation in target sites. Additionally, positions of high variation among sRNAs in a superfamily are shown to correlate with variable nucleotide positions in the target site: a sign that *Cuscuta* small RNAs may be selected to target numerous hosts. We conclude that trans-species active small RNAs are present and functional in all of the *Cuscuta* species we've examined to date, but that their sequences have diverged substantially. This raises the hypothesis that trans-species active small RNAs may be a common, but quickly evolving feature within the *Cuscuta* genus.

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[P28] Finding a good decoy for *Striga hermonthica* suicidal germination

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Striga hermonthica non hosts stimulate parasite seed to germinate without getting infected because the non-hosts produce unique germination stimulants (strigolactones). This phenomenon called suicidal seed germination is greatly used in *S. hermonthica* control. For improved efficiency of the suicidal seed germination method in *S. hermonthica* control, detailed analysis of comparative ability of potential trap crops to stimulate germination is critical. An additional and often ignored determinant of suitability of non host intercrops is the extent to which the parasite can penetrate various non hosts and if such interaction can adversely affect the trap crop. This is critical because although the parasite is not able to effectively infect a non host, it may still cause injuries that can expose the crop to other pathogens such as bacteria and fungi. In this study, we determined the suitability of Kenya's commonly used legumes (cowpea, pigeon pea, common bean, and garden pea) as potential intercrops in the control of *S. hermonthica*. We firstly determined their efficiency to induce germination of *S. hermonthica* seeds using germination assays. We then assayed the amounts and types of strigolactones in their root exudates using a high performance liquid chromatography coupled to tandem mass spectrometer (LC-MS/MS). Finally, we determined the extent of interaction between the legumes and *S. hermonthica* using histological analysis. We found no significant differences (p -value = 0.0001) with regard to the legumes efficiency to induce *Striga* germination. Analysis of strigolactones in root exudates of legumes revealed that the most abundant strigolactones were 2-epi-5-deoxystrigol and orobanchol with trace amounts of 2-epi-orobanchol and strigol. Expectedly, non of the legumes fully supported growth and development of *S. hermonthica* to enable the parasite complete its lifecycle. However, the extent of parasite penetration varied greatly in the different legumes. Cowpea and garden pea formed vascular connections with *S. hermonthica* parasite. However, the parasite did not grow beyond pigeon pea's endodermis, and in the common bean, the parasite barely attached on the host cortex. Our findings suggest that all legumes tested are appropriate for use as intercrops because they induced high *S. hermonthica* seed germination. Additionally, confounding effects (if any) on successful penetration of *S. hermonthica* in non-hosts will require further investigation.

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[P29]The role of hormones in parasitic plant infection

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Parasitism in plants has evolved independently more than eleven times. Despite independent origins all parasitic plants form an invasive organ termed the haustorium. The haustorium forms in the presence of a compatible host, penetrates and connects to the hosts vasculature to withdraw water and nutrients. Previous studies have identified host root exudates that act as haustoria inducing factors (HIFs). HIF perception leads to reactive oxygen species accumulation in certain parasites and is considered to be an early signal for haustorium formation initiation. To date little is known about the developmental processes activated after HIF perception leading to haustorium formation. Here we investigate the role of plant hormones on early haustorium formation in the facultative root parasite *Phtheirospermum japonicum*. Various plant hormones were exogenously applied to in vitro *Phtheirospermum japonicum* infection assays. Our experiments showed that gibberellic acid application led to an increase in haustoria number. On the contrary, the application of a gibberellic acid biosynthesis inhibitor led to decrease in haustoria number. Furthermore, the application of cytokinin ceased haustoria formation resulting in underdeveloped haustoria that did not penetrate the host. Experiments investigating the interaction between gibberellic acid and cytokinin showed that cytokinin application could suppress the effects of gibberellic acid, indicating that cytokinin is dominant over gibberellic acid. Together these results suggest an antagonistic interaction of gibberellic acid and cytokinin in early haustoria development where gibberellic acid promotes and cytokinin opposes haustoria formation.

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[P30] Plant-to-plant interactions: cytoskeleton of stem and root parasitic plants

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Plant cytoskeleton, comprising microtubules, actin filaments and cytoskeleton-associated proteins, is largely involved in the regulation of plant morphogenesis as well as in plant responses to both abiotic and biotic environmental challenges. Parasitic plants, together with herbivores, pathogenic fungi, oomycetes, parasitic nematodes, bacteria and viruses, emerge as a key biotic invaders able to trigger the reorganization of host cytoskeleton in the due course of hypersensitive response and system-acquired resistance. However, only recently the organization of both stem and root parasitic plants cytoskeleton was revealed for the first time by immunolabeling techniques and fluorescence microscopy. Generally, the cytoskeleton in parasitic plant shoots is organized in a similar way to non-parasitic dicots, while it has some peculiarities in structures specific for the parasitic lifestyle.

Dodders (*Cuscuta*) are stem parasites with the specific organs – root-like structure, physiologically senescing at 7th–10th day postgermination in case of the successful establishment of vascular connection with the susceptible host, and lateral prehaustorium/haustorium – the connecting bridge initially required for the pirating of water and nutrients from the host. Many dividing cells present in dodder's shoot apical meristem, in contrast to root-like structure, which is devoid of cell divisions. Cortical microtubules are oriented transversely and/or obliquely, while actin filaments are randomly distributed in cells of both organs. Furthermore, longitudinal cortical microtubules are present in digitate cells of prehaustorium, and transverse arrays – in its file cells. Long and short random actin filaments are also observed in prehaustorium cells.

In turn, holoparasitic root parasites from widely distributed and economically devastating genera *Striga*, *Orobanche* and *Phelipanche* germinate after an exogenous application of the synthetic strigolactone GR24 and/or the host root exudates. In actively elongating germ-tubes, palillae, prehaustorium, haustorial hairs, and intrusive tip cells a specifically oriented net of cortical microtubules, individual actin filaments and long cables of F-actin are present.

The understanding of cell mechanisms including those involving cytoskeleton is important for the development of the efficient protective approaches against highly invasive parasitic weeds.



[P31] Natural resistance to witchweed in cultivated and wild finger millet accessions

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Striga spp. is a noxious parasitic weed that infects major cereal crops like sorghum, maize, millet and rice, causing severe damage that may result in up to 100% yield losses in Sub-Saharan Africa. Little research has been done to establish the effect of *Striga* on finger millet, even though *Striga* damage has been on the rise in the last decade. The utilization of host-based resistance has been limited in finger millet due to the lack of durable resistant sources within cultivated varieties. To understand the genetic variation for *Striga* resistance in finger millet, we screened both wild and cultivated finger millet genotypes against *Striga hermonthica* using laboratory, screenhouse and field assays. We also used microscopic and histological analysis to establish the extent of haustorium invasion of the plant cells upon infection. Our results suggest more durable resistance within wild finger millet in comparison to cultivated accessions. Histological analysis revealed different extents of resistance responses in early days of parasitism (3 DAI). In the landrace (Okhale-1), *S. hermonthica* did not manage to penetrate host tissue beyond to endodermis and therefore no vascular connections between host and parasite resulted. In the wild finger miller (LESK 10), the parasite made vascular connections-albeit in less frequency. Field experiments undertaken across three locations confirmed the glasshouse and laboratory results as well as reports from other host cereal crops, which implicate a mechanical barrier and/or incompatible response against *S. hermonthica* parasitism. The resistant wild finger millet genotypes can now be integrated into breeding programs to improve genetic resistance in farmer-preferred varieties.

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[P32]Elucidating Strigolactone Biosynthesis in *Zea mays* (maize)

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Strigolactones are plant hormones regulating a.o. shoot branching, root architecture and secondary stem growth, and are also exuded from the roots into the rhizosphere where they serve as host detection signals for beneficial arbuscular mycorrhizal (AM) fungi. On the other hand, strigolactones have been hijacked by parasitic plants, members of the Orobanchaceae, as germination signal to start their parasitism. Parasitic plants such as *Striga* are a major threat to maize cultivation in the African continent and are difficult to control. In maize root exudate, two canonical strigolactones - 5-deoxystriol and sorgomol - have been reported (Yoneyama et al., 2015). Recently, we demonstrated the occurrence of seven new strigolactones in maize root exudate, of which two were structurally characterized as zealactone (Charnikhova et al, 2017) and zeapyranolactone (Charnikhova et al, 2018). The role of these structurally different strigolactones in *Striga* infection and/or other biological processes is unknown. Unraveling their biosynthesis pathway should help elucidating this.

Therefore, as a first step, we identified the genes encoding the core pathway of strigolactone biosynthesis, up to carlactone, based on the homology with the core pathway in other species (Alder et al, 2012). Agroinfiltration of these core pathway genes (D27, CCD7 and CCD8) in *Nicotiana benthamiana* resulted in the production of carlactone.

Subsequently, candidate genes involved in the downstream strigolactone biosynthetic pathway were identified using co-expression analysis using the identified core strigolactone biosynthetic pathway genes as bait. A combination of heterologous expression (transiently in *Nicotiana benthamiana* and yeast), metabolomics and mutagenesis approaches are used to elucidate the downstream steps in the biosynthetic pathway of strigolactones in maize and study the biological relevance of the structural diversity in these (rhizosphere) signaling molecules.

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[P33]Transformation and regeneration protocol of parasitic plant *Phelipanche ramosa*

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Parasitic plants belonging to the genera *Phelipanche* and *Orobanche* (broomrapes) are economically important parasitic weeds, causing considerable crop yield losses. Despite extensive research on the interaction of *Phelipanche* spp. with their hosts, mechanisms to control germination and development of these parasites have not been identified yet. Targeting molecular and biochemical processes involved in the establishment of the connection between the parasite and the host may offer a new perspective for control. The development of effective transformation and regeneration protocols for parasitic plants is essential for functional studies of parasite genes that are considered to be involved in the parasite-host interaction. However, parasitic plants are difficult to transform and regenerate due to their specific life cycle. *Phelipanche* and *Orobanche* spp. are holoparasites that need to attach to the roots of a host plant to get their assimilates, nutrients and water to develop and reproduce.

In the present study, we show a new highly efficient *Agrobacterium rhizogenes*-mediated transformation protocol for *P. ramosa* (*A. rhizogenes* MSU440) carrying a non-destructive selection marker gene coding for a red fluorescent protein (DsRed1). Using this protocol up to 90% transformation efficiency was obtained. Examination of whole developed transgenic *P. ramosa* plants under the fluorescence microscope proved, that the DsRED signal was present in all parts. We consider this plant as fully transgenic and plant development was not influenced by presence of the DsRed gene. Moreover, for the first time, we describe shoot and flower development of *P. ramosa* in vitro in the absence of a host plant.

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[P34] Trophic specialization and host-related diversification in the Broomrape family (Orobanchaceae)

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The Broomrape family (Orobanchaceae) is the most important group of parasitic plants. Its enormous species diversity, which includes some of the most noxious parasitic weeds, spans the entire range of trophic specializations. Distributed all over the world (excluding Antarctica), the parasites also exhibit a great array of ecological preferences. However, the evolutionary mechanisms and causes underlying lifestyle specializations as well as host preference and life history transitions remain unclear. Here, we established a phylogenetic framework for 355 species covering all trophic specialization of Orobanchaceae and compiled data about their ecological preferences and biogeographical ranges. Our phylogenetic analyses reveal a deep phylogenetic incongruence between nuclear and plastid data. While the latter support a sister relationship between the witchweed (Buchnereae) and the broomrape (Orobancheae) clade, nuclear data resolve a grade in which Orobancheae are sister to a grade of Rhinanthae, Pedicularideae, and Buchnereae. Integrating over both phylogenetic and trait uncertainty, we estimated the distribution of convergent host preference and distribution regimes across the family using a priori-free modeling under the maximum likelihood paradigm. Further, we reconstructed the evolution of ecological traits over the phylogenetic trees. Our results suggest that the last common ancestor of all recent parasitic Orobanchaceae was a facultative hemiparasite with an annual lifespan. Transitions into obligate parasitism occurred multiple times independently, resulting in at least five losses of (effective) photosynthesis. Our models also suggest that a reversal from obligate to facultative parasitic lifestyle is unlikely. Life histories change frequently along both trophic specialization and host preference transitions, and we observe that the dependence on germination stimulants might have been gained (or lost?) independently within the family. Our study provides a first Orobanchaceae-wide reconstruction of the evolution of ecological preferences and biogeographical range expansions, potentially enabling us to improve predictions of the weed potential of selected Orobanchaceae species.



[P35] Genome sequencing of branched broomrape and reconstruction of the evolutionary history of the *Phelipanche ramosa*/*P. aegyptiaca* species complex

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The holoparasitic *Phelipanche ramosa* and *P. aegyptiaca* (Orobanchaceae) belong to the most important parasitic weeds. Generating genomic resources for these broomrape species is hampered by their huge genomes, conservatively estimated to be over 4 Gbp. Here, we present the first genome draft for *P. ramosa* alongside a phylogenomic analysis of the *P. ramosa*/*P. aegyptiaca* complex. Based on 10X linked-read, Nanopore long-read, and Illumina short-read data, we obtained a pseudo-haploid assembly of 3.81 Gbp lengths. A BUSCO analysis indicates a level of completeness comparable to that of *Mimulus* and *Solanum*, although several conserved genes remained undetected in *P. ramosa*, perhaps due to natural gene losses associated with the holoparasitic lifestyle. Our first annotation, assisted by newly generated transcriptome data for three belowground and two aboveground developmental stages, finds 31,848 gene models. Of these, ca. 20 % cannot be assigned to an orthogroup, and 1,673 genes cluster in 51 *Phelipanche*-specific orthogroups. Matching the *P. ramosa* gene models to existing data of *P. aegyptiaca* shows minimal genetic differentiation between both species. Further, a comprehensive gene family analysis with nearly a hundred other flowering plants provides us the basis for a robust inference of lifestyle-related changes of gene content in broomrape. Shallow resequencing of populations covering several species, whose actual status as independent species must be debated, show little genetic differentiation between non-weedy and weedy populations within the *P. ramosa*/*P. aegyptiaca* species complex. Taxon clusters are somewhat concordant with geographic origin, implying local adaptation. Both maximum likelihood and Bayesian inferences suggest that some weedy populations have arisen from populations that are confined to non-crop hosts, whereas some non-weedy populations appear to have evolved from species or populations that prefer to infest agricultural crops. Together, the newly sequenced genome of *P. ramosa* and our analysis of the evolutionary history of the *P. ramosa*/*P. aegyptiaca* species complex open the door for future functional-genetic and ecological-evolutionary study of broomrape.



[P36] Microbial disruption of the signalling between Sorghum and the parasitic weed Striga

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Recently, a paradigm shift in the plant sciences emerged in which microbiomes are seen as a functional driver of plant growth and health. Microbiomes can substantially expand the genomic and metabolic capabilities of plants, providing essential functions including nutrient acquisition, modulation of immune responses and enhanced (a)biotic stress tolerance. However, there is still scarcity in the literature with respect to the potential of soil and plant-associated microbiomes in disrupting the lifecycle of plant parasitic weeds. Particularly the root parasitic weed *Striga*, which is the major biotic constraint to food production in Africa, severely affecting smallholder farmers and food security. We aim to characterize the mechanisms by which microbes can suppress *Striga* and modulate its interaction with the host *Sorghum bicolor*. We screened a collection of microbes, comprising 146 strains of various bacterial genera, using a newly developed machine learning software to quantify germination, for their ability to disrupt the molecular signalling between *Sorghum* and *Striga* in pre-defined phenological stages of the parasites lifecycle. These constitute the initial phases of the infection, that is, the germination of *Striga* seeds and the formation of the haustorium (parasitic structure). We identified several bacteria from different phyla that significantly suppress *Striga* seed germination via microbial volatile organic compounds (mVOCs) profiled using GC/Q-TOF. We generated the complete genome sequence of all isolates and are focused on identifying genomic regions responsible for the suppressive phenotype. Ultimately, we aim to integrate the genomics data with the metabolomics data to identify putative biosynthetic gene clusters for the volatiles resulting in a suppressive phenotype. Our work provides new insight into the yet unknown metabolic and genomic potential of soil and plant-associated microbes and makes progress in identifying the mechanisms and microbial metabolites involved in the suppression of root parasitic weeds.



[P37] Does introgression between two parasitic plants have local adaptation advantages for their offspring?

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Rhinanthus major and *R. minor* are two annual plant species that are known to hybridize in nature. In naturally mixed populations of these two hemiparasitic species, most of the advanced-generation hybrids found are close to *R. major*. This is likely due to its higher attractiveness to the pollinating bumblebees, so introgression of genetic material takes place from *R. minor* into *R. major*. We have hundreds of diagnostic SNP markers for both species, which we obtained using ddRADseq. The detected SNP markers will be used to analyze advanced hybrids from naturally mixed populations of these two species in different habitats. We will compare the patterns of introgression and identify similarities and differences in introgressed markers among the populations. Presence of SNP markers from *R. minor* with a high frequency in advanced hybrids in all populations will help us to find genomic regions with a general fitness advantage for these hybrids. *R. minor* SNPs that are absent or found in low frequencies in the studied populations are likely to be linked to loci with disadvantageous alleles for hybrid fitness. SNP markers with a high frequency in some habitats and absent or in low frequency in other habitats are indicative of introgressing alleles that only confer a fitness advantage in certain habitats. The results of this first screening will be presented.

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[P38]Molecular detection of the *Striga* seedbank in Ethiopian soils

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Controlling the *Striga* seedbank is one of the major challenges in the management of this devastating parasitic weed in agricultural fields in Sub-Saharan Africa. Hence, fast, high-throughput and robust techniques for the in situ detection and quantification of the *Striga*-seedbank are needed. Such techniques have paramount importance to assess the impact of different intervention strategies on *Striga* seedbank and to unravel the association between soil microbiological and physicochemical properties and *Striga* seedbank dynamics. Here, we designed RT-qPCR primers that amplify specific *Striga* genes. Both in silico and in vivo tests showed that the primer is specific to *Striga* seed. The efficiency of the technique was assessed in proof-of-principle experiments involving introduction of known numbers of *Striga* seeds (9 and 243 seeds) in *Striga*-free Dutch agricultural soil samples with contrasting soil physicochemical properties. We used a K₂CO₃ density-based approach to separate *Striga* seeds from other organic debris and soil particles. After grinding and DNA extraction, RT-qPCR analysis showed that as little as 9 *Striga* seeds per 100 g of soil could be detected and quantified. This new technique is now adopted to screen 48 sorghum field soils collected over 2800 km from different agroecological zones in Ethiopia. Moreover, the associations between the *Striga* seedbank, soil physicochemical properties, *Striga* field infestation, management practices and microbiome composition of these Ethiopian soils will be presented.

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[P39]Ethylene-mediated host responses increase resistance to *Phelipanche aegyptiaca*

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The polyethylene (PE) bag system is a useful method to nondestructively observe attachment and growth of root parasitic plants on host roots (Goldwasser et al., 2002), but over years of experience growing *P. aegyptiaca* in this system we noticed that excess nutrient solution at the time of attachment inhibits *P. aegyptiaca* growth. We hypothesized that decreased parasite success might be related to ethylene induction produced by high water levels because flooding conditions are known to induce ethylene levels in plants (Grichko and Glick 2001). To test this, we compared the number of *P. aegyptiaca* tubercles on Arabidopsis roots under optimum or excess liquid situations and found that excess liquid reduced the number of tubercles by 50% compared to the lower moisture control condition. Use of Arabidopsis plants with the ethylene-inducible GUS reporter system (EBS::GUS) (Stepanova et al., 2007) revealed that ethylene responses in the Arabidopsis EBS::GUS roots were induced by conditions of excess liquid or parasitism by *P. aegyptiaca*. Constitutive ethylene activation mutants of Arabidopsis and tomato, such as constitutive triple-response (CTR) mutants (Broekgaarden et al., 2015), have increased tolerance to *P. aegyptiaca*, suggesting that host production of ethylene is associated with enhanced resistance to parasitism. We also found that the application of an ethylene precursor (ACC) to host roots decreased attachment of *P. aegyptiaca* by 80% as compared to the non-treated controls. These results help explain the performance of parasites in the PE bag system and support the idea that ethylene-mediated defense responses can increase resistance of host plants to *P. aegyptiaca*.

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[P40]*Cuscuta campestris* Jasmonate-Induced Protein 23 (CcJIP23) is a functional mobile protein.

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We are increasingly aware of the exchange of macromolecules such as RNAs between *Cuscuta* and their hosts, but the functional significance of such transfer remains unclear. Recognizing that proteins are important functional molecules regardless of their site of synthesis, we sought to investigate mobile proteins in the parasite-host interaction. We explored the *Cuscuta campestris* / *Arabidopsis thaliana* system using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) to analyze total protein extracts from host and parasite stems near the haustorium region. From three biological replicates we identified 97 mobile *Cuscuta* proteins in *Arabidopsis* stems and 447 mobile *Arabidopsis* proteins in *Cuscuta* stems. Among the most highly abundant *Cuscuta* mobile proteins found in the host was a *Cuscuta campestris* 23 kDa Jasmonate-induced protein (termed CcJIP23; Andresen et al., 1992). The mRNA encoding this protein was found in previous transcriptome analyses as mobile from *Cuscuta* to *Arabidopsis* and tomato hosts (Kim et al., 2014). JIP23 has not been well characterized in other plants, so we set out to identify the CcJIP23 function using yeast two-hybrid (Y2H) screening and *Arabidopsis* transgenic (overexpression and silencing) plants. Y2H screening revealed that CcJIP23 interacts to *Arabidopsis* l-3-ketoacyl CoA thiolase (AtKAT2) which is involved in the jasmonate synthesis pathway (Castillo et al., 2004). *Arabidopsis* transgenic plants over-expressing CcJIP23 (35S::CcJIP23) flowered earlier than control Col-0 plants, which may be explained by elevated transcript levels of *Arabidopsis* flowering locus T (AtFT) in the 35S::CcJIP23 plants. In addition, *Cuscuta* growth on *Arabidopsis* transgenic plants silencing the CcJIP23 was lower than growth on control plants. In sum, we hypothesize that *Cuscuta* produces CcJIP23, which may be mobile as a transcript, protein, or both, moves into the host and acts to regulate the jasmonate pathway of the host plant, resulting in suppressed defense response against *Cuscuta*. CcJIP23 is thus a novel *Cuscuta* effector protein.

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[P41]Message received: Evidence for translation of mobile mRNAs in *Cuscuta*-host interactions

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Messenger RNAs (mRNAs) readily move between *Cuscuta* and its hosts, forming a remarkable example of interspecies sharing of nucleic acids (Kim et al. 2014). This exchange is potentially a mechanism for the parasite or host to influence growth of - or gain information from - the interacting plant, but the function of mobile mRNAs remains unclear in the parasite-host association. One possible fate of mobile mRNAs is translation into protein after reaching the recipient organism, with the resulting protein potentially affecting the destination cell. We have taken a multi-pronged approach to exploring the possibility of mobile mRNA translation, including use of reporter genes encoded by mobile mRNAs, TRAP (Translating Ribosome Affinity Purification; Zanetti et al., 2005), and proteomics. First, we studied *Arabidopsis* plants that express versions of the GUS reporter gene with or without a mRNA mobility-conferring motif (Zhang et al., 2016), and found that the host-to-*Cuscuta* movement of GUS mRNA was correlated with the appearance of blue in the parasite stems, indicating GUS enzyme activity. We verified that the GUS protein itself did not appear to be mobile in this system, but additional evidence is needed. A second approach to evaluating translation was conducted using TRAP, in which epitope tagged *Arabidopsis* ribosomes facilitated the purification of ribosomal complexes and the presence of specific bound *Cuscuta* mRNAs. Finally, we assayed protein content of *Arabidopsis* and *Cuscuta* growing in association with each other, and found that hundreds of host and parasite proteins are present in the corresponding non-self organism. Based on the large size and insolubility of some proteins, as well as results from ¹⁵N labeling experiments, we conclude that some proteins were likely synthesized in the destination organism. Taken together, our results point to a complex *Cuscuta*-host interaction in which proteins from one species are found in another as a result of either direct protein mobility or translation of mobile mRNAs. The function of mobile proteins in this system requires further research.

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[P42] Identification and characterization of QTL underlying resistance in rice to the parasitic weed, *Striga asiatica*

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Striga asiatica is an obligate hemi-parasitic weed, that poses a serious threat to rain-fed cereal production in sub-Saharan Africa. Limited understanding of the identity and mode of action of genes underlying *S. asiatica* resistance in rice hinders the breeding of resistant rice varieties. This work aims to characterise a post-attachment resistance interaction between an accession of *S. asiatica* from Ethiopia (Sa-Eth) and two rice lines with different levels of resistance —IR64 which is susceptible to Sa-Eth and CT8556-37-2-3-1-M which is more resistant. To understand the underlying genetics of the phenotype, quantitative trait locus (QTL) analyses using a population of recombinant inbred lines (RILs) derived from the two rice lines were carried out.

The resistance phenotype was characterized over a period of six weeks in a soil-free rhizotron system. The number, biomass and individual length of Sa-Eth growing on five replicates per each of the two rice lines were compared at different time points after inoculation of the roots with germinated Sa-Eth seeds. There were no significant differences between the numbers of *S. asiatica* growing on the two rice lines over the time course; however, the *S. asiatica* individuals growing on CT8556-37-2-3-1-M accumulated substantially less biomass at a slower rate than those on IR64. Moreover, only a few of the *S. asiatica* individuals growing on CT8556-37-2-3-1-M reached the larger class sizes, unlike majority of the *S. asiatica* growing on IR64. We concluded that the resistance phenotype was associated with a slower rate of growth and development of *S. asiatica* in contrast to other types of resistance where the parasites die soon after attachment.

In order to understand the genetic basis of the phenotype, 178 RILs of the mapping population —which had been genotyped by sequencing — were phenotyped in nine batches with four replicates per line, randomly assigned to the batches. Rice roots in rhizotrons were inoculated with germinated Sa-Eth seeds. The *S. asiatica* biomass at 28 days post inoculation per root system was used to quantify the level of susceptibility of each RIL. QTL analyses were carried out in MapDisto, R/qtl, QGene, QTL Cartographer and IciMapping software packages using simple interval, composite interval, inclusive composite interval and multiple QTL mapping. Three QTL for *S. asiatica* resistance were identified on chromosomes 1, 5 and 11. Genomic studies are underway to identify candidate genes within the QTL regions underlying the difference in growth and development of the parasites.



[P43] Assessing the tolerance of the advanced putative mutant rice lines M5 generation (*Oryza sativa*) to *Striga hermonthica* attack

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The parasitic weeds of the genus *Striga* cause yield losses in cereal crops such as rice, maize and Sorghum in Africa. The aim of this study was to assess the reaction of the advanced putative mutant rice lines M5 generation tolerant to *Striga asiatica* face to *Striga hermonthica* attack at the tolerance level. Pots experiments were performed in the greenhouse. Thirty-eight advanced mutant rice lines of the M5 generation selected as putatively tolerant to *S. asiatica* with the parent sensitive Var. B22 from Madagascar were used. These putative mutant rice lines called “PMRL” and the parent were evaluated in 3 treatments whose: PMRL+*S. asiatica* seeds; PMRL+ *S. hermonthica* and PMRL *Striga* free as negative control. Each treatment was replicated four times in the pot containing mixture of soil-sand inoculated by the viable *Striga* seeds. The number of emerged *Striga* plant per pot, the host plant height and the percentage damage were measured and recorded. The tolerance index was calculated from the host plant height. The results have shown that the data indicated significant differences between the treatments and tested lines whatever the variable considered. The number of emerged *S. asiatica* was significantly lower than those of the *S. hermonthica*. The percentage damage was significantly lower $9.16 \pm 5.33\%$ to $31.59 \pm 3.27\%$ for the PMRL+ *S. asiatica* than those of the PMRL+ *S. hermonthica* $79.16 \pm 4.16\%$ to $100.00 \pm 0.00\%$, the parent + *S. asiatica* is $57.46 \pm 0.92\%$ and the parent + *S. hermonthica* is $95.83 \pm 4.16\%$. The tolerance index is higher, varies from 0.93 to 0.74 for the PMRL+*S. asiatica* and then 0.61 for the parent+ *S. asiatica* versus 0.11 to 0.26 for the PMRL+ *S. hermonthica*, then 0.15 for the parent+ *S. hermonthica*. One rice line was found resistant to *Striga hermonthica*. These results suggest that the tolerance level of these advanced putative mutant rice lines is very low face to the attack of *S. hermonthica*. The gene responsible for the resistance mechanism to *Striga* in these lines cannot be the same for *S. asiatica* and *S. hermonthica*.

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[P44] Does silicon enhance rice resistance to the root parasitic weed, *Striga hermonthica*, and the Egyptian cotton leafworm, *Spodoptera littoralis*?

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Silicon is a quasi-essential element for plants. Rice, alongside other grasses, is adapted to accumulate silicon in high concentrations, which provides benefits such as protection against many above- and below-ground abiotic and biotic stresses. The root hemi-parasitic weed *Striga hermonthica* is a major constraint to cereal crop production in Africa and amending of soils with silicon could provide a relatively low cost and sustainable way of enhancing resistance to these devastating parasites. Given that rice accumulates approximately 90% of its silicon into shoots, amending soils with silicon may have the additional benefit of enhancing above-ground defences in rice, for example, against larval feeding by species from the genus of polyphagous moths *Spodoptera littoralis*, which cause additional constraints to crop production in Africa. In some areas, both *S. hermonthica* and *S. littoralis* co-occur on the same crops.

In order to investigate the hypothesis that increased silicon uptake will increase below-ground resistance to *S. hermonthica* and above ground resistance to *S. littoralis*, rice plants were grown in pots containing sand with or without the addition of silicon in the form of silicic acid. In addition, the effect of silicon and *S. hermonthica* infection on rice leaf resistance to *S. littoralis* was investigated.

Whilst silicon treatment did not affect rice resistance to *S. hermonthica*, silicon treatment increased rice resistance to *S. littoralis*. Infection of rice roots by *S. hermonthica* increased rice leaf silicon concentration (in the absence of supplied silicon) to that of silicon-treated rice plants. However, this increase did not enhance rice leaf resistance to feeding by *S. littoralis*, suggesting that the physical presence of silicon in rice leaves was not the only determining factor of resistance to feeding by *S. littoralis*. These results prompt future work to investigate the potential indirect effects of *S. hermonthica* on above-ground defences in rice.



[P45] Wood radial and axial variation of some *gigantica Loranthaceae* mistletoes

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From a hydraulic perspective, mistletoes show one of the most interesting examples of xylem structure. These plants can develop a “chimeric sap transport system”, which comprises cells of both host and parasite wood. Additionally, these parasites may cause an overload for the sap transport capacity, since they usually bear stomata in both sides of the leaf, showing poor gas exchange control. Therefore, the capacity to regulate and adjust the transport system is a key factor in determining the probability of survival of a parasitic plant. This work investigated whether and how the wood formation of some mistletoes varies within the stem topology. Three Loranthaceae species were selected for this study, all possessing relatively large dimensions, either radially or axially and occurring naturally in Brazil. *Psittacanthus robustus* is a woodrose-forming shrub with thick branches up to 3m long and 4 cm in diameter; *Tripodanthus acutifolius*, a lianescent/tree species that reaches several meters long; and *Struthanthus flexicaulis*, a decurrent shrub with very flexible and long stems (up to 4 m). The length of both vessel elements and fibres was observed to stabilize about 2mm away from the pith. Thus, our data suggest that at least for the thickest stems (*P. robustus* and *T. acutifolius*), the secondary xylem indeed reaches the state of mature wood in relatively thin diameter, which makes the comparison with other species viable. In the case of *Struthanthus flexicaulis*, there is no clear change of pattern along the stem axis. So far, our data suggest that one of two things might be occurring: i) there is a reduction on the mean vessel diameter along the stem, which would lower the risk of cavitation by raising the resistance of that part of the system; ii) there is an increase in the total vessel density, which generates both an increase of conductivity of the system, but primordially its redundancy. Both of those characteristics would ultimately serve the same objective: to increase the hydraulic safety in the parts that theoretically undergo highest hydraulic tension. Furthermore, changes in cell size were observed in a periodical fashion. In short, these plants seem to possess a very plastic xylem, capable of adapting its structure and organization to better absorb nutrients from the host without killing it.



[P46]Comparative wood anatomy of Brazilian mistletoes genera of Loranthaceae

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Wood anatomy probably is one of the most important aspects for the understanding of the success of mistletoes, being responsible for maintaining a resilient and efficient transport system from the connection to the host to the parasite's crown. In spite of that, wood anatomical features of mistletoes are poorly known. Loranthaceae is one of the most diverse parasitic groups, comprising both root and stem hemiparasites, and gathering the largest number of genera within the Santalales order. 12 of these genera occur naturally in Brazil, most of them comprised in the subtribe Psittacanthineae, distributed in a variety of habitats, biomes and, therefore, ecological niches. Here we aimed to study the wood anatomy of Loranthaceae species by developing a comparative study of all Brazilian genera, trying to summarize what may be an anatomical profile for the family. Wood material were obtained from both living organisms and from herbaria and xylaria. So far, *Psittacanthus*, *Tripodanthus*, *Struthanthus* and *Gaiadendron* species were analyzed. Our results suggest species in this family could have enough wood-structural similarities so that a common anatomical profile of the parasitic stem wood. These plants show great diversity regarding vessel lumen size (≤ 50 up to ≥ 200 μm) within the same species, with common vessel dimorphism. Vessel elements occur in many types of groupings, but mostly in clusters, with wider vessel elements associated with very narrow ones, and/or tracheids, providing an efficient water uptake, as well as redundancy and safety of the hydraulic system. Other common characteristics include well-developed banded parenchyma; very thick-walled fibres and tracheary elements; and distinguished radial system with tall rays. All these traits seem to be associated with supporting the xylem by lowering the risks of embolism and possibly aiding in the refilling of vessels, traits commonly reported for specimens under very low hydric potentials. All these features are possibly associated with a change of habitat from root to stem parasitism, given that most of them are not shared with the tree species *Gaiadendron punctatum*, one of the three genera of root parasites that supposedly have diverged earlier in the family.

APG, 2016. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV; *Botanical Journal of the Linnean Society* 181: 1-20 pg.



[P47] Exploring the evolutionary origin of haustorium development in root parasitic plants

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The Orobanchaceae root parasitic plants develop haustoria in response to host root exudates. Similar mechanisms are proposed to regulate the interactions of non-parasitic plant roots as well [1]. Changes in cis-regulatory elements are thought to contribute to phenotypic divergence more than protein changes because of the higher pleiotropic effects associated with altering the coding sequence [2]. Therefore, we hypothesize haustorium development may have been co-opted to an earlier root-root recognition mechanism through changes in the cis-element regulation of autotrophic plant genes and pathways. To investigate this, I am comparing the promoters of haustoria related genes from Orobanchaceae parasites to identify cis-elements regulating the transcriptional changes in response to host root exudate. We created an RNASeq dataset for the first 24 hours of haustorium development in the hemiparasite *Triphysaria versicolor* to identify groups of genes co-expressed during haustorium initiation and early development. Promoters for these genes were obtained through sequencing the *T. versicolor* genome using a 10X Genomics library preparation and the Illumina NovaSeq platform. The promoters of genes with similar transcriptional changes are being compared for over-represented cis-elements that may be responsible for their co-regulation. In addition to this approach, we are using phylogenetic footprinting to identify cis-elements conserved between Orobanchaceae species. We collected eighteen species of annual hemiparasitic Orobanchaceae and are comparing the promoters of orthologous genes with similar host induced expression to find conserved cis-elements. The cis-elements identified through both methods are being assayed for host induced spatio-temporal activity using a fluorescent protein system. By characterizing the transcriptional regulation of genes involved in haustorium initiation and early development in Orobanchaceae parasites, it can identify the changes needed for haustoria to originate from a non-parasitic plant.

1. Depuydt, S, 2014. Arguments for and against self and non-self root recognition in plants. *Front. Plant Sci.* 5, 614.
2. Wittkopp, P. J. & Kalay, G, 2011. Cis-regulatory elements: molecular mechanisms and evolutionary processes underlying divergence. *Nat. Rev. Genet.* 13, 59-69.



[P48] Aberrant protein phosphatase 2C leads to ABA insensitivity, high transpiration rate and sustenance of parasitism in *Striga hermonthica*

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Striga hermonthica parasitizes major crops including sorghum, millet and maize in sub-Saharan Africa. *Striga* infection causes devastating losses in yield and the damage is more serious under drought conditions. High transpiration rate of *Striga* helps to maintain a gradient in leaf water potential towards the parasite and facilitates the diversion of water and solutes to the parasite. We found that *Striga* seeds and stomata in leaves did not respond to abscisic acid (ABA), the phytohormone responsible for controlling seed dormancy and stomatal closure in plants. Protein phosphatase 2C of *Striga* (ShPP2C1) was not regulated by ABA receptors, and this feature was attributable to specific mutations in its amino acid sequence. *Arabidopsis thaliana* transformed with ShPP2C1 showed an ABA-insensitive phenotype, indicating that ShPP2C1 functions as a dominant negative regulator of ABA signal transduction. These findings suggest that ShPP2C1 interrupts ABA signaling in *Striga*, resulting in high transpiration and a subsequent flux of host nutrients and water from the host to the parasite. Moreover, we found accumulation of a large amount of ABA in host-free *Striga* plants and seedlings. A considerable portion of ABA produced by the seedlings was exuded. The phytohormone in the rhizosphere could, at least in part, contribute to the bewitching effects, wilting and chlorosis, displayed by *Striga*-infested plants even before the parasite emerges from soil.

Fujioka, H., H. Samejima, H. Suzuki, M. Mizutani, M. Okamoto, and Y. Sugimoto, 2019. Aberrant protein phosphatase 2C leads to ABA insensitivity and high transpiration in parasitic *Striga*. *Nature Plants* 5: 258-262
Fujioka, H., H. Samejima, M. Mizutani, M. Okamoto and Y. Sugimoto, 2019. How does *Striga hermonthica* bewitch its hosts? *Plant Signaling & Behavior* 14



[P49]Development of propiconazole derivatives as strigolactone biosynthesis inhibitors

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Strigolactones (SLs) are carotenoid-derived small molecules that have been recognized as one group of the plant hormones regulated various growth and development such as shoot branching and root development. SLs were originally discovered as seed germination stimulants for root parasitic weeds. Root parasitic weeds, such as *Striga*, *Orobancha*, and *Phelipanche* spp., inflict severe damage on agricultural production especially in sub-Saharan Africa, the Middle East and Asia. To date, definitive control methods have not been established. Because SL deficient mutants protect against the infection of root parasitic weeds, SL biosynthesis inhibitors have a potential to control damage from root parasitic weeds by preventing seed germination. We were previously performed screening for SL biosynthesis inhibitors to find azole derivatives that reduced SL content in exudation from rice root. Through this screening experiment, propiconazole fungicide was selected as a likely inhibitor of SL biosynthesis. In this study, we evaluated propiconazole as SL biosynthesis inhibitor in detail. As propiconazole is registered as pesticide, its use will reduce the cost for the development of regulators of root parasitic weeds. We first measured the level of 4-deoxyorobanchol (4DO), a major endogenous SL in rice, in root and root exudates using the LC-MS/MS. Propiconazole showed 4DO inhibitory activity in a dose-dependent manner within the concentration range of 1-30 μ M both in root and root exudates. The biological activity of these compounds was evaluated by examining elongation of second tiller bud and dwarfism. Propiconazole induced the outgrowth of second tiller bud and inhibited the length of rice seedling at 1 μ M. The compound-induced bud outgrowth and dwarfism were partially restored by the co-application of 1 μ M GR24, a SL analog. We checked the *Striga hermonthica* germination rate of the root exudates from propiconazole-treated rice. In accordance with the results of the 4DO level in root and root exudates, the culture media of propiconazole-treated rice showed less germination stimulating activity than those of mock-treated.

To optimize activity and selectivity, propiconazole derivatives were synthesized. Some derivatives inhibited the level of 4DO in exudation from rice root more strongly than propiconazole. These results suggest that propiconazole derivatives should affect SL biosynthesis. Further analysis are needed to clarify the target enzyme and specificity of these compounds as SL biosynthesis inhibitor.



[P50]What's in a name? That which we call a haustorium by any other name would cause misunderstanding of parasitic plant biology

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The parasitic life form in plants is associated to the formation of an organ generically called haustorium, which is responsible for the connection between parasite and host. The great diversity of parasitic species - about 1% of living angiosperms, added to the diversity of potential hosts results in a multiplicity of structures, tissues and cell types peculiar to these interactions. However, it is often observed that all this morpho-anatomical variety is approached under the use of few common terms and even with synonyms, but also with ambiguous terminological use and uncertain homology. The use of publications originally written in other languages than English (i.e., mainly Spanish, French, Italian, and German), often being literally translated, dealing with false cognates and other linguistics influences have also caused some confusion. In order to promote a clearer and more precise use of terminology, in addition to performing a historical retrieval of original meanings, a compilation of terms was carried out. The compilation process was initially based on a literature review containing a glossary, previously published by Job Kuijt in 1977, from which 33 terms and their respective synonyms were selected. To these, 15 other entries were added based on more recent publications. Once selected, each term was analyzed regarding its used and original conception in order to promote a better understanding of the nomenclature used. In addition, anatomical and ontogenetic studies have been compiled from the literature and/or carried with fresh or preserved biological collection material, then re-analyzed under a phylogenetic perspective. A total of 48 terms are presented and discussed in this illustrated and referenced glossary, with which we suggest a standardization and an update of more adequate terms for the description of the parasite-host interface in all parasitic lineages. The haustorium of parasitic plants should be understood as a complex organ, such as a root or a stem, which is composed of different tissues and cell types. The frequent equalization of this complete organ with one of its parts could lead to misunderstandings of the very parasitic nature of some species and lineages of parasitic plants. In addition, we highlight the need for caution when comparing structures formed by severely modified and reduced plants (such as endoparasites) with those of free-living species. Further developmental studies analyzing these plants should broaden the current comprehension of their structure and evolution.

Aber, M., A. Fer, and G. Sall. 1983. Etude du transfert des substances organiques de l'hôte (*Vicia faba*) vers le parasite (*Orobanche crenata* Forsk.): Transfer of Organic Substances From the Host Plant *Vicia faba* to the Parasite *Orobanche crenata* Forsk. *Zeitschrift Pflanzenphysiologie* 112 (4): 297308. [https://doi.org/10.1016/S0044-328X\(83\)80047-6](https://doi.org/10.1016/S0044-328X(83)80047-6).



[P51]Scaling up of Integrated Striga Control in Sorghum in Ethiopia

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Striga spp are major challenges to production of cereal crops including sorghum. Yield losses up to 100% are common in sorghum growing areas and arable lands are often abandoned because of the prohibitive parasite populations. The “Integrated Striga Control (ISC) in Sorghum in Ethiopia and Tanzania” Project was developed and implemented by Purdue University in collaboration with EIAR, Amhara, Oromia, SNNP and Tigray Bureaus of Agriculture in Ethiopia from 2012 to 2016. The overarching goal of the project is to improve income, food security and livelihood of small scale sorghum farmers by promoting and disseminating ISC technologies. The major components of the project include deployment of officially released Striga resistant and drought tolerant varieties, multiplication and distribution of seed and conducting a large number of ISC demonstration plots on farmers’ fields. Seed production and its distribution were conducted by the federal and regional research institutes, seed enterprises, private farms and cluster of farmers in Oromia, Amhara, Tigray and SNNP regions. A total of 15 tons and 120 tons of foundation and certified seeds of Striga resistant and drought tolerant sorghum varieties were produced, respectively, from 2012-2016. ISC package demonstrations and popularization of Striga resistant varieties were conducted on a total of 18,000 farmers’ plots and on more than 500 farmers’ associations (kebeles). The project mobilized diverse stakeholders, integrated various disciplines and covered large production areas. Significant improvements in the production and distribution of high quality seeds of improved Striga and drought tolerant varieties was achieved. Concerted efforts in the training and technology transfer activities has created high demands for Striga resistant varieties that needs to be met to further boost production and improve food security in Striga prone regions.



[P52]miPEPs: new tools to study and control *Orobanche cumana*

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The broomrape species *Orobanche cumana* causes important losses to the production of sunflower in countries surrounding the Black Sea, in Southern Europe and in growing area of France. Unfortunately, no efficient and sustainable methods to control this parasitic weed are currently available. The objectives of the miPEPiTO project are to develop new molecular tools to investigate the biology of the parasite, and to develop an innovative and sustainable biocontrol technology for management of this pest. One partner of the project has recently discovered a new class of regulatory peptides, the miPEPs (miRNA-encoded peptides), which are encoded by primary transcripts of miRNAs. Each miPEP stimulates the transcription of its own encoding transcript, leading to the production of higher amount of the corresponding miRNA and consequently to a downregulation of specific target genes. This natural molecular regulation of gene expression can be obtained with synthetic miPEPs, so that specific stages of plant development can be perturbed temporally by exogenous treatment with appropriate miPEPs.

We aim to decipher the roles of miRNAs in the biology of the pathosystem *O. cumana*-sunflower, by using exogenous treatments of sunflower and/or *O. cumana* miPEPs; and, to test the hypothesis that some identified miPEPs may negatively affect this pathosystem by decreasing broomrape growth and infection, and by improving sunflower resistance.

To date, thanks to the availability of the *O. cumana* genome, the miRNA repertoire of this parasitic species has been identified. It consists of 42 members grouped in 16 families. Every target gene sequences (43 in total) of these miR genes were identified and their expression analyzed during a period of 48 hours following a GR24 treatment of the seeds. Forty two miPEPs were also identified and produced, among which 23 inhibit *O. cumana* seed germination. Interestingly, the member a of the miPEPun1 showed the strongest inhibiting effect while its member b did not. Two out of the four corresponding miRun1 target genes exhibited an up regulation following a GR24 treatment, which was impaired by a miPEPun1a treatment. This down regulation of expression by miPEPun1a is associated to increase of the relevant pri-miRun1a expression. Analysis of miPEPs activities on haustorium development is in progress.

These initial results are promising and indicate that miPEPs should allow use to increase our knowledge on key molecular mechanisms underlying a complex parasitic interaction and that they should provide a new phytosanitary method to control broomrape parasitism with highly specific and biodegradable natural substances.

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Lauressergues, D., J.M. Couzigou, H.S. Clemente, Y. Martinez, C. Dunand, G. Bécard and J.P. Combier, 2015. Primary transcripts of microRNAs encode regulatory peptides. *Nature* 520: 90–93

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[P53] Lifecycle synchronization between parasitic plants and their hosts assessed by molecular evolutionary analysis and host-induced gene silencing

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The Broomrape family (Orobanchaceae) contains the worst agricultural weeds. While we know that many obligate parasites of Orobanchaceae rely on host-exuded molecules for their earliest development, it remains unknown how far lifecycle synchronization extends and whether it includes reproduction. Here, we analyze the molecular evolution and function of gene complexes involved in flowering time regulation of Orobanchaceae. Specifically, we aim to test whether the induction of flowering is an autonomous process in holoparasitic plants and if shifting from annual to perennial hosts leaves selectional footprints in the parasites' flowering time regulators. Using a custom bioinformatic pipeline, we searched 25 Orobanchaceae species for 12 flowering time genes, upstream and downstream of the central inductor. The resulting alignments were subjected to molecular evolutionary analysis, for which we used branch-site and trait-rate fusion models under the maximum likelihood paradigm. Further, we used these data to design primers for parasite-specific RNA-silencing constructs, which allows us to target different flowering pathways of *Phelipanche ramosa* and *P. aegyptiaca* via host induced gene-silencing. Following the successful transformation, we will then assess phenotypic changes in flowering time and duration through rhizotron and pot assays. We are confident that our combination of molecular evolutionary analyses of genes sets from a broad range of parasites and functional analysis can uncover the mechanisms underlying the control of parasite flowering.



[P54]Structural and functional analysis of haustorium inducing factors for the parasitic plant *Striga hermonthica*

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Striga hermonthica is a parasitic plant that belongs to the Orobanchaceae, and parasitizes important crops such as rice, maize and sorghum. *Striga* causes big damages to agriculture with billion US dollars of estimated annual yield losses. *Striga* forms a specific organ called a haustorium at its radicle tip to infect their host plants. Haustorium is induced by host-derived chemicals called haustorium inducing factors (HIFs). 2,6-dimethoxy-p-benzoquinone (DMBQ) is one of known HIFs isolated from root extract of a host plant *Sorghum*. It was suggested that DMBQ is produced from host-derived syringic acid by oxidation-reduction reaction using reactive oxygen species (ROS) and peroxidase. However, it remains unknown how DMBQ induces a haustorium and how it behaves in a plant. In this study, we aim to understand properties and action mechanisms of HIFs for haustorium induction in *Striga*.

At first, we investigated the effects of various ROS inhibitors for haustorium induction in *S. hermonthica*. We found that when H₂O₂-scavenging enzyme catalase was treated on *Striga*, haustorium formation rates were decreased in both DMBQ- and syringic acid-induced haustoria, suggesting that H₂O₂ is necessary at downstream of DMBQ. Moreover, haustorium formation rates were reduced by treatments of NADPH oxidase inhibitors and peroxidase inhibitors. From this result, we conclude that NADPH oxidases and peroxidases were indispensable for haustorium formation in *Striga* (Wada et al., 2019). Next, we examined the changes in amounts of DMBQ and syringic acid during *Striga* haustorium induction. When syringic acid was added on culture media of *Striga*, the amount of syringic acid in the culture media was decreased and DMBQ was increased. This result suggests that *Striga* can accelerate the conversion of syringic acid to DMBQ. When we added DMBQ on the culture media of *Striga*, the amount DMBQ in the culture media was decreased until under detection limit within 24 hr. Interestingly, however, this culture media retains high haustorium inducing ability, despite that DMBQ was under detection limit in the solution. This implies presence of unknown HIF compounds in the culture media.

In parallel, we are screening a chemical library for identification of HIFs and haustorium formation inhibitors. Structure-activity relationship analysis revealed a common structure for haustorium inducing activity. In this presentation, we will discuss structural requirement and downstream events of HIFs for haustorium induction in *Striga*.

Wada, S., Cui, S., and Yoshida, S. (2019). Reactive oxygen species (ROS) Generation Is Indispensable for haustorium formation of the root parasitic plant *Striga hermonthica*. *Front. Plant Sci.* 10, 1–12. doi:10.3389/fpls.2019.00328.



[P55]Biology and management of *P. aegyptiaca* in cabbage

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Cabbage (*B. oleracea* var capitata), a biennial crop belonging to the Brassicaceae family. In Israel alone 2,200 hectares of cabbage are been cultivated. Egyptian broomrape (*Phelipanche aegyptiaca*), a member of the Orobanchaceae family, is a parasitic plant that considered as a main troublesome weed in vegetables and field crops around the world and causes severe yield loss. Broomrape germinates and attaches to the cabbage roots in responds to chemical compounds that are exudes from the cabbage roots to the rhizosphere. Once established, the broomrape becomes a major sink that draw all its growth requirements from the host resulting with crucial damage to the host plant. One of the most effective ways to manage broomrape in the field is applying herbicides. In this work two herbicides (glyphosate and ethametsulfuron-methyl) were examined for crop selectivity and broomrape control efficacy in cabbage at the sub-surface broomrape developmental stages. The main objective of this research is to develop a decision support system for broomrape management in cabbage, using the following sub-objectives: (1) characterizing the parasitism dynamics in the soil sub-surface stages; (2) detecting herbicides which will not harm the cabbage but will be toxic for the attached broomrape; (3) characterizing the cabbage root exudates which stimulates the broomrape germination. In addition to growing the cabbage with the broomrape in different temperature regimes in growth chambers, we employed minirhizotron camera, which allows non-disturbing observations upon the broomrape development in situ (in the soil subsurface in the field), to characterize the broomrape parasitism dynamics. Another objective is to compare different varieties of cabbage for and examine their susceptibility to different broomrapes collected from different hosts. Our results show that (1) glyphosate under a rate of 72 gr ha⁻¹ and ethametsulfuron methyl in all rates cause no harm to the cabbage but effectively controlled Egyptian broomrape. (2) *P. aegyptiaca* seeds that were collected from cabbage infected field germinate on cabbage roots in higher rate than *P. aegyptiaca* seeds that were collected from *P. aegyptiaca* infected to other crops. *P. aegyptiaca* germinate at the presence on the cabbages roots between in temperature range of 12-300C. However, the optimal temperature growth for the host and the parasitic is (day/night) 24/180C.



[P56] Transcriptional changes in tomato roots upon P starvation and the role of strigolactones in this response

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Phosphorus (P) is an essential macronutrient for plant growth and productivity of crops. Strigolactones (SLs) are mainly produced in the roots of plants and have been shown to play an important role in the root and shoot architectural changes under low P conditions (Al-Babili and Bouwmeester, 2015). However, the role of SLs in the transcriptional response of plants under P deficiency is unknown. A transcriptome analysis of tomato roots under P starvation and P replenishment was performed to get a better understanding of the general response of plant roots to P stress. A total of 58, 331, 406 and 187 significantly differentially expressed genes (DEGs) were identified upon 2, 3, 4 and 5 days of P starvation compared with control roots. This shows that tomato rapidly responds to P starvation and reaches the highest level of response after 4 days. Gene functional analysis of these DEGs showed that glycolipid synthesis, phospholipid degradation and sucrose phosphate synthase were all induced by P deficiency and repressed by P replenishment, suggesting that remodeling of both lipid and sugar metabolism are two essential adaptive mechanisms to P stress in tomato. Interestingly, the upregulation of these DEGs under P starvation was reduced in a SL biosynthetic mutant, carotenoid cleavage dioxygenase 8 (*ccd8*) (Kohlen et al., 2008). In addition, Mapman analysis suggests that SLs are required for the changes in the expression under phosphate starvation of many genes involved in secondary metabolism (isoprenoids, flavonoids, phenylpropanoids and phenolics) as well as plant hormone metabolism (ABA and GA). Taken together, these results reveal that SLs play a crucial role in the remodeling of plant metabolism upon P starvation.

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[P57] *Triphysaria* controls vegetative self-recognition by restricting release of HIFs in roots

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Plants secrete and respond to a wide array of chemicals to communicate in rhizosphere (Massalha et al. 2017). Root parasitic plants develop haustoria in response to haustoria inducing factors (HIFs) released by neighboring host plants (Yoshida et al. 2016). The ability to distinguish self from non-self in root parasitic plants presumably maximizes their beneficial connections with hosts. *Triphysaria* is a hemiparasite in Orobanchaceae and has a broad host range, while it rarely parasitizes other *Triphysaria* (Yoder 1997). We hypothesized that the chemical differences between host and parasite root exudates might contribute to the vegetative self-recognition phenomenon in *Triphysaria*. Bioassays of root exudates from three *Triphysaria* species and two host species (*Arabidopsis* and *Medicago*) showed that *Triphysaria* seedlings only formed haustoria with exudates from host roots but not parasite roots. One known HIF DMBQ was eluted in the most active fraction of *Arabidopsis* root exudate in HPLC. Quantification of DMBQ concentration in each root exudate via LC-QQQ-MS/MS revealed that the two host plants secreted similar levels of DMBQ while parasite root exudates contained no or very little amount of DMBQ. These results suggested that the difference in HIF concentration between host and parasite root exudates may account for the vegetative self-recognition in *Triphysaria*. Previous studies reported DMBQ could be produced from phenolic compounds by phenol oxidases. By exogenously applying commercial phenol oxidases (horseradish peroxidase and fungal laccase) to intact *Arabidopsis* and *Triphysaria* roots, we found that functional HIFs were released from both host and parasite roots, but DMBQ was released only from *Arabidopsis* roots. We have generated multiple transgenic *Triphysaria* lines to constitutively overexpress *Arabidopsis* peroxidase and fungal laccase genes *in vivo* and we are currently phenotyping these transgenic roots to find out if self-recognition in *Triphysaria* could be partially lost. Our results suggested that HIFs could be produced from *Triphysaria* roots by phenol oxidases and that *Triphysaria* might restrict the spacial or temporal expression of proper phenol oxidases, such as peroxidases or laccases, in order to prevent self-parasitism. In the long term, our study of self-recognition in parasitic plants may be useful agriculturally by applying similar strategies into crop plants to make them invisible to parasitic weeds.

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[P58]Eco-evolutionary causes and consequences of parasitism in plants

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Parasitism is a successful way of life, one that is ubiquitous to all organismal domains. In plants, the transition from a free-living organism to being a parasite that steals water and nutrients either from another plant or from mycorrhizal networks provides a significant ecological-evolutionary advantage in highly competitive and resource-poor environments. The transition of plants to a nonphotosynthetic lifestyle brings about extreme morphological changes and massive genetic reconfigurations. Here, we explore the progression of the so-called parasitic reduction syndrome on the genetic level and elucidate how parasitism affects diversification patterns. The natural diversity of parasitic specializations and phylostatistical hypothesis testing revealed that already the establishment of obligate parasitism triggers genomic reconfigurations. After the eventual loss of photosynthesis, functional and physical reduction proceed rapidly predominantly in plastid genomes, accompanied by a gradual acceleration of molecular evolutionary rates. The transition to obligate parasitism apparently triggers an eco-evolutionary feedback loop that affects all genomic compartments, which might further trophic specialization (Wicke et al. 2013, 2016). As this happens, we observe that diversification patterns change en route to holoparasitism. We hypothesize that parasite speciation and diversification is co-shaped by an increasing dependency on their host plants, which creates an isolating barrier absent from ordinary plants (Maul et al. 2018).

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[P59] Inoculation of rhizobia enhanced the damage of holoparasitic plant on host plant which will not be affected by exogenous nitrogen

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Interspecific interaction has been shown to have strong effect on plant community and even ecosystem. Little study was found to explore the stem holoparasite-host interactions include below-ground communities. Here, we conducted a common garden microcosm experiment with soybean, parasitic *Cuscuta australis*, mutualism rhizobia and exogenous nitrogen test the host plant mediated multiple trophic interactions between parasitic plant and root mutualist, and their influence factors. Inoculation of rhizobia and addition of nitrogen significantly increased photosynthetic rate of soybean, while parasites significant decreased. Addition of nitrogen significantly shifted the effect of rhizobia on photosynthesis. Inoculation of rhizobia weakened the deleterious effect of parasites, while addition of nitrogen enhanced the effect of parasites on the photosynthesis of soybean. Parasites significant decreased the plant height, leaf number, below-ground biomass, above-ground biomass and total biomass, while inoculation of rhizobia significantly increased them. Inoculation of rhizobia enhanced the deleterious effect of parasites on host soybean. Inoculation of rhizobia significantly increased the number of haustoria, circles number, and the total biomass of parasites, while addition of nitrogen significantly increased them, however, no interactive effect was found between inoculation of rhizobia and addition of nitrogen on the growth of parasites. Parasites significantly decreased the dried weight of rhizobia and addition of nitrogen enhanced the deleterious effect of parasites on the number and dried weight of rhizobia (significantly interactive effect of nitrogen and parasites).

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[P60]Dual transcript profiling of plant-plant interaction between parasitic weed *Orobanche cumana* and sunflowers

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Orobanche cumana is an obligate root parasite specifically infecting sunflowers in agricultural fields and have caused severe yield and economy losses worldwide. For efficient control strategies, it is necessary to understand defense mechanism of host sunflowers and pathogenesis of *O. cumana* during their interactions at molecular levels. Here, we used the dual RNA-seq approach to simultaneously assess the transcriptomes of two contrasting sunflowers and *O. cumana* during their interactions. Infection of *O. cumana* triggered defense responses in sunflowers, which was insufficient during susceptible interactions as compared to resistant interactions, probably due to the failure of recognition of parasite effectors. Secretome prediction from *O. cumana* transcriptome enabled robust identification of proteins associated with *O. cumana* penetration and infection. Vigorous secretion activities of *O. cumana* were observed during susceptible interactions, with 181 secreted proteins predicted. Further analyses showed that these proteins were involved in cell wall degradation, pathogenesis, nutrient acquisition and hormone-related proteins. Remarkably, infection of *O. cumana* imbalanced host hormone biosynthesis and signaling, and enhanced nutrients efflux from host through utilization of own transporters and inhibition of assimilation and/or transport of nutrients for host use. In summary, our findings provide deep insight into the mechanisms underlying *O. cumana* infection and make progress on the knowledge of interactions between host plants and parasitic plants.

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[P61] Characterization of germination stimulants for *P. ramosa*

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Phelipanche ramosa causes severe damages to oilseed rape (*Brassica napus*) in southern France. Our previous study demonstrated that 2-Phenyl isothiocyanate (2PEITC) is an active germination stimulant for *P. ramosa* and found in root exudates of oilseed rape. Then, 21 different ITCs were examined for *P. ramosa* seed germination stimulation and some important structural features of ITCs for exhibiting *P. ramosa* seed germination stimulation have been uncovered. We further modified ITCs and obtained some ITCs that are highly active to *P. ramosa*. All the ITCs examined did not induce germination of *Orobancha minor*. These results demonstrate that ITCs are important germination stimulants for *P. ramosa* and thus *P. ramosa* has developed a special seed germination strategy to parasitize oilseed rape, a non-host of arbuscular mycorrhizal fungi and may not exude canonical strigolactones.

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[P62]Induced mutation in Sorghum for Striga-resistance

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Sorghum bicolor (Linn.) Moench is the staple crop in terms of cereal production and per capita consumption in Burkina Faso. Sorghum production is highly affected by *Striga hermonthica* which is a major biotic constraint. This study aims at inducing Striga-resistance in popular indigenous sorghum varieties while maintaining the good qualities of the parents. Dry seeds of two sorghum varieties Sariaso14 and S 29 were irradiated at 200, 300, 400 and 500 Gy. The irradiated seeds and controls were sown. M1 panicles were harvested, and planted as M2 panicle-to-one progeny. M2 plants were selected and advanced to M3 and then M4 generations using pedigree selection method based on plant vigor, tiller number, early maturity and grain and panicle types as selection criteria. M4 mutants of Sariaso14 (derived from the treatments 96/200Gy, 120/300Gy, 122/400Gy and 164/500Gy and one ICSV1049 mutant derived from the treatment (84/500Gy) were screened for Striga resistance in rain-fed cropping conditions. Each Sorghum family M4 was therefore planted on one row 8 m length with 1 m between planting hills. Sorghum hills were artificially infested with at least 103 Striga seeds/hill-1 at planting. As results, a phenotypical diversity was observed within the population. Appearance of new characters such as the shape of stalk, the sheet and the color of the grain were observed with the M5 lines. The analysis of variance for the effects of the four doses of gamma irradiation on Sorghum plants M4 revealed high fluctuations of induced variability in the number of useful tillers (CV% = 723, and 300 for M4 lines of Sariaso 14 and S29), the panicle weight (CV% = 131 and 48 for M4 lines of S 29 and Saiaso 14), and the rate of panicle filling (CV%=84 and 48 for M4 lines of S29 and Saiaso14). The productive M4 lines in sorghum grains came from Sariaso 14 and S 29 seeds irradiated with 200 Gy and 400 Gy, respectively. With regard to Striga-infection, no Striga plants emerged around the hills of 3 of M4 plants of which one S 29 mutant and two Sariaso14 mutants. Field experiments will be repeated along with bioassays before multi-location evaluation.

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[P63]Effect of salinity on *Cuscuta campestris* parasitism

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Parasitic plants are no less affected by abiotic stress than any other plant. In addition to the direct effects, however, abiotic stress factors may induce physiological changes in the host, which further influence the parasites. These include alteration of germination stimulants, e.g. strigolactones, transmission of harmful substances from the host to the parasite, activation of defense mechanisms of the host and decrease of available host biomass (Frost et al., 2003; Hassan et al., 2010; Vurro et al., 2011). Moreover, parasitism may add additional pressure to host plants in combination with abiotic stress, impairing their ability to successfully cope with challenging environmental conditions (Cochavi et al., 2018). We aimed to study the influence of salinity, being one of the most adverse abiotic stressors, to the parasitism of *Cuscuta campestris* and its interactions with host plants. First, salinity lead to significant decrease of germination rate and growth in host absence, already significant at 100 mM NaCl and it also negatively affected the ability of successful establishment and haustoria formation. Further growth after infection was also affected by salinity, but in a host-dependent manner. While *Arabidopsis thaliana* and *Capsicum annuum* were shown as worse hosts under salinity, opposite trend was observed in *Thymus vulgaris* and *Medicago sativa*. The pre-parasitism stages of *C. campestris* lifecycle were shown as more sensitive to salinity, while establishment on a suitable host guaranteed further development of the parasite. Under salinity, the parasite also tended to accumulate higher than the host concentrations of the osmoprotectant L-proline, while no significant changes in antioxidant enzymes were observed (Zagorchev et al., 2018). Accordingly, *Cuscuta* parasitism impaired the regular salt stress response of the host *Arabidopsis* plants. This effect, in terms of proline accumulation and antioxidant response was shown not only in the direct site of infection (e.g. infected leaves), but also in distant host tissues non-infected leaves and roots. In conclusion, our results suggest that although not a typical halophyte, *C. campestris*, which is widely accepted as a parasitic (and invasive) weed with high ecological plasticity (CABI, 2019) could successfully thrive in saline conditions, assuming that suitable host plants are available. Furthermore, salinity alters the host susceptibility to the parasite and parasitism affects salt stress response of the host, both of which are substantial threats for natural plant societies and agriculture under climate changes.

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[P64]Phylogenetic and functional analyses of parasitism genes in haustorial formation and development

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The Parasitic Plant Genome Project (PPGP) is dedicated to understanding genetic changes associated with parasitism by sequencing the transcriptomes of three related parasitic species (*Triphysaria versicolor*, *Striga hermonthica*, and *Phelipanche aegyptiaca*) in the Orobanchaceae. We hypothesize that genes with elevated expression in the haustorium and/or the haustorial interface in multiple related parasites are most likely to play essential roles in parasitism. Results from the PPGP have generated a set of forty gene orthogroups common to all three parasites, called “core parasitism genes”. To analyze the potential functions of parasitism genes, and identify targets for disruption of parasite-host interactions, we narrowed down the gene list to a number of tractable candidates by careful analysis of homology and stage specific gene expression. As a result, thirteen orthogroups containing evolutionarily conserved parasitism genes were selected. Orthologous genes from several recently published parasitic plant genomes and transcriptomes, such as *Cuscuta campestris*, *Thesium chinense*, and *Phtheirospermum japonicum*, were integrated into gene family research pipelines to gather a broader insight into parasitism gene evolution across multiple parasitic lineages. To better understand the evolutionary history of the chosen genes and select for the most essential parasitism genes for further investigation, the Galaxy PlantTribes tools (https://galaxyproject.org/tutorials/pt_gfam/) were used to construct SuperOrthogroup phylogenies, which contains the target orthogroups and other closely related ones. Our phylogenetic analysis also provides preliminary evidence of gene family expansion, signatures of adaptive evolution, and the presence of chimeric genes in certain gene families. Among these, the Pectate Lyase (PL) gene family was of special interest, since it contains an Orobanchaceae specific clade with haustorial expression. To study the involvement of PL in parasitic plant invasion, the model parasitic plant, *Triphysaria versicolor*, was transformed with hairpin RNAi constructs targeted against TrVePL1, a PL that is specifically upregulated in the haustorium. Seven days post inoculation with a host, a greater proportion of failed haustoria was observed in transformed root cultures. A similar result was achieved by transforming the same construct into the host plant, *Medicago truncatula*, indicating the potential for using host-induced gene silencing (HIGS) to knock down parasitic plant genes and actively reduce colonization success of parasitic plants. We are extending this research into *Striga* and *Phelipanche*. Functional studies of parasitism genes in the model parasite *T. versicolor* will give us a deeper understanding of parasitic biology, and the success in HIGS will lead to the development of new methods to reduce parasitic invasion.



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