

## PROGRAM OF ORAL PRESENTATIONS

### **2000 Years of Observation, Knowledge and Research on *Orobanche***

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A historical survey will be presented on *Orobanche*, beginning with the first observations and descriptions, followed by the period of collecting and classification of *Orobanche* species in Linn's taxonomic system. *Orobanche* species have been collected in herbaria or they were preserved in alcohol (Racovita in Romania). *Orobanche* species up to date would have remained botanical curiosities among the wildflowers, if not some of them turned aggressive and became agricultural pests. The aim of *Orobanche* control became the driving force for intensive agricultural research. Agricultural problems also led to the organization of special *Orobanche* workshops besides the International Parasitic Weeds Symposia. When plant physiologists and plant biochemists became interested in *Orobanche* /host systems and *Orobanche* development increasing complexity became evident. Important steps in revealing the sophisticated systems will be emphasized, some problems in understanding *Orobanche* life will be pointed out. Moreover, the question will be asked, why most *Orobanche* species die back and need to be protected, why they have not become agricultural pests. What is the future of *Orobanche* research?

## **Horizontal Gene Transfer Gone Wild in Parasitic and Other Flowering Plants.**

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I will review the published evidence and present unpublished data showing that there has been considerable transfer of mitochondrial genes between parasitic plants and their host plants during angiosperm evolution. This transfer has occurred in both directions, from host to parasite and vice-versa. A model will be presented explaining why transfers can involve very large regions of the mitochondrial genome, why mitochondrial gene transfer is relatively frequent, and why chloroplast gene transfer is never seen.

**Complete DNA Sequences of the Plastid Genomes of Two Parasitic Flowering Plant Species, *Cuscuta reflexa* and *Cuscuta gronovii***

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We have sequenced the complete plastid chromosomes of two parasitic flowering plant species, *Cuscuta reflexa* and *Cuscuta gronovii*. Both species are capable of performing photosynthesis, though with different efficiencies. Together with the achlorophyllous *E. virginiana*, of which the plastid genome was already published over a decade ago, these species represent a progression series towards total dependency on the host plant. The plastid chromosome sizes were found to be larger than that of *E. virginiana* with 121.5 kbp and 86.7 kbp, respectively. Although the chromosome structure is similar to that of non-parasitic plants, a number of insertions, deletions (indels) and sequence inversions were found. Gene losses are more pronounced in *C. gronovii* than in *C. reflexa*, encompassing some photosynthetic genes as well as many regulatory genes. Transcription of the *C. reflexa* ptDNA resembles that of nonparasitic higher plants regarding promoter structure and transcription patterns but shows marked differences in the steady state RNA profiles. In *C. gronovii*, genes for the photosynthetic apparatus were retained while the *rpo* genes coding for the plastid-encoded RNA polymerase that is responsible for their transcription were lost. Concomitantly, adaptations within the plastid genome have occurred that enable transcription mediated exclusively by a nuclear-encoded plastid RNA polymerase. Moreover, *C. gronovii* is the first plant where a loss of the intron-encoded splicing factor MatK was observed. Thus, *Cuscuta* is an interesting model for the evolution of reduced organellar genomes.

### **Phylogeny and Evolution of *Orobanche* and Related Genera (Orobanchaceae)**

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In recent years, molecular phylogenetic studies have greatly enhanced our understanding of the phylogenetic relationships of *Orobanche* and related genera. These data suggest splitting *Orobanche* into (at least) four segregates in agreement with previous suggestions based on morphological and karyological evidence. These genera show partly different evolutionary trajectories, e.g., incidence of polyploidy or diversity and evolution of retrotransposons. Irrespective of taxonomic consequences, the phylogenetic framework allows rigorous testing of character evolution, e.g., the evolution of genome size or the correlated evolution of host range and life span. Additionally, *Orobanche* and related genera may show features of general interest, such as the occurrence of horizontal gene transfer.

## **The Evolutionary Origins of Aerial Parasitism in Santalales**

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Molecular phylogenetic work in the sandalwood order (Santalales) has progressed to the point where multiple DNA sequences of nuclear and plastid genes are available for nearly all of the 146 genera. Moreover, phylogenetic methods have matured with the development of model-based methods such as maximum likelihood (ML) and Bayesian inference (BI) that more effectively deal with rate heterogeneity - an issue with some parasitic plant lineages. Past work showed that aerial parasitism evolved five times independently in Santalales, but the relative timing of these diversifications was not addressed. DNA sequences from five genes were obtained from 39 taxa representing all families in Santalales. These data were analyzed using ML and BI and time estimates were performed with a Bayesian relaxed molecular clock and penalized likelihood methods. The trees were calibrated using published fossil data from Santalales and an outgroup. These analyses showed that aerial parasitism first arose in Misodendraceae ca. 89 Mya and subsequently in Viscaceae (81 Mya), "Eremolepidaceae" (62 Mya), tribe Amphorogyneae in Santalaceae (53 Mya), and Loranthaceae (30 Mya). The rapid adaptive radiation and speciation in Loranthaceae coincides with the appearance of savanna biomes during the Oligocene. Reconstructing the character "parasite mode" on the tree suggests that all lineages except Misodendraceae evolved from ancestors that were polymorphic for either root or stem parasitism. Features found in more derived aerial parasites, such as the squamate habit, unisexual flowers, and loss of chlorophyll, have evolved independently and thus represent convergences and parallelisms.

## **Reticulate Evolution in the Parasitic Genus *Cuscuta* (Dodders; Convolvulaceae)**

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The frequency and relative importance of hybridization in plants has been an area of intense debate. Although this evolutionary mechanism has received considerable attention from plant biologist, there are no well-supported cases of reticulate evolution involving holoparasitic plants to date. Recent molecular phylogenetic analyses revealed that the subgenus *Grammica*, the largest and most diverse group of the stem parasitic genus *Cuscuta*, consists of 15 major clades. We describe here five cases of strongly supported discordance between phylogenies derived from plastid and nuclear data and interpret them as results of five independent hybridization events. Three of these cases probably represent relatively recent reticulations because each of them involves more closely related species, always confined within the same major clade as their putative parental species, and are sympatric/parapatric in distribution with them. In contrast, the two remaining cases involve species whose potential progenitors are derived from different major groups of *Grammica* and are allopatric in their present distribution. This is consistent with more ancient hybridization events. Exemplary taxa illustrating these two cases (i.e., *Cuscuta* subsection *Denticulatae* and *Cuscuta sandwichiana*, respectively) are discussed in more detail.

### **Speciation and Host Specificity in *Orobanche***

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*Orobanche* (broomrapes) are obligate parasites which attach to the roots of other plants as seedlings. Each species has a preferred host-range and while some species parasitize variety of plants, others are much more host-specific. It is possible that speciation in parasitic plants, like *Orobanche*, might be triggered by a shift in host preference. Continued divergence between parasite populations could then be driven by ecological isolation and/or selfing. We speculate that changes in host preference could be triggered by hybridization if hybrids possessed transgressive phenotypes that pre-adapted them to parasitism of a new host. We are investigating this hypothesis by studying an *Orobanche* complex in the UK in which two species, *O. minor* and *O. picridis* merge, and intermediate morphotypes, possibly hybrids between them, occur. The parent species' distributions are restricted to where their preferred hosts occur, while putative hybrids appear to occupy a new niche on coastal turf. Preliminary Inter-simple sequence repeat marker data shows evidence of introgression, suggesting that intermediate morphotypes are hybrids. We are also using rhizotrons (a bioassay in which host and parasite are cultivated) to investigate the host preferences of *O. minor*, *O. picridis* and the putative hybrids to determine whether hybridization can cause a change in host preference. Ultimately our goal is to determine whether hybridization and shifts in host specificity contribute to speciation in parasitic plants.

## **Non-Stimulated Spontaneous Germination of *Orobanche* is Genetically Controlled**

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A fundamental step in the evolution of the angiospermous root holoparasites is their ability to germinate only in response to stimulants that are released by host roots. Germination away from a host leads to seeding death. We assumed that the trait requiring stimulant receptivity evolved by the development of genes that inhibit germination, and switch off inhibition upon chemical stimulation. By analyzing inter-specific hybrid populations of *Orobanche cernua* and *Orobanche cumana*, segregating for spontaneous germination that occurs without chemical stimulation, we identified genes that are involved in germination control. The segregation results correspond to the expectation that two epistatic genes are responsible for this germination control. Our model suggests that one root parasite dormancy gene (*Rpdg1*) is a regulator of non-stimulated germination, with a dominant negative control on the expression of *Rpdg2* gene. We hypothesize that *Rpdg1* is involved in seed dormancy, and *Rpdg2* in the reception or transduction of the germination stimulus. For spontaneous germination a seed should express the recessive alleles of *Rpdg1* and the dominant allele of *Rpdg2*. Our results agree with the 3:13 (spontaneous vs non-spontaneous germination) ratio in the first segregating generation, which matches to our assumption that one parent species possess the two dominant genes, and the other has the two recessive genes. These results indicate that spontaneous germination of *Orobanche* is genetically controlled. Such genetic control ensures a low incidence of this lethal trait among natural parasite populations, and increases the rate of its successful germination in the vicinity of host roots.

**Qualitative and Quantitative Differences of Strigolactone Exudation Determine Host Specificity of Root Parasites *Orobanche* and *Striga***

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Strigolactones, originally identified as germination stimulants for root parasitic weeds, *Striga*, *Alectra*, and *Orobanche*, are now recognized as important chemical signals for arbuscular mycorrhizal (AM) fungi symbionts. Even non-mycotrophic plants like spinach exudes strigolactones, indicating that strigolactones may have some other important functions in plants and/or in rhizosphere communities. In addition, plants appear to exude mixtures of strigolactones along with other unknown germination stimulants, probably including non-strigolactones. It is likely that the amounts and composition of these stimulant mixtures may vary with plant species, their growth stages, and their growth conditions including nutrient availability. Furthermore, for example in sorghum, different cultivars exude different mixtures of strigolactones. These results suggest that quantitative and qualitative differences of strigolactones have some effects on host recognition by AM fungi and root parasitic weeds. Examples of qualitative and quantitative differences of strigolactones among host and non-host plants will be presented and discussed in relation to host-recognition by AM fungi and root parasites.

### **Strigolactones, Signals for Friends and Enemies**

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The strigolactones are signaling molecules that are used by the root parasitic broomrapes (*Orobancha* spp) and witchweeds (*Striga* spp) as well as the symbiotic arbuscular mycorrhizal (AM) fungi to detect the presence of their plant host. The requirements for such signaling molecules are that these compounds should be specifically indicative of the presence of a plant host. We are elucidating the biosynthetic origin of the strigolactones in several host plants of AM fungi as well as parasitic plants. This led to the discovery that the strigolactones are derived from the carotenoids - and should therefore be called apocarotenoids and not sesquiterpene lactones - and as such are quite “plant-specific”. Considering the dual signaling role of the strigolactones it is interesting that there is an interaction between AM fungi and parasitic plants through the host plant, resulting in reduced parasitism by *Striga hermonthica* on sorghum and maize that are colonized by AM fungi. We will discuss the evidence that this interaction is mediated through the production of strigolactones. Also the further elucidation of the strigolactone biosynthetic pathway, initially focusing on the first dedicated step that is probably catalyzed by a carotenoid cleavage dioxygenase will be discussed. We are cloning genes from the pathway to make transgenic plants with altered strigolactone biosynthesis. These plants will be a great tool to study the importance of these signaling molecules for the interaction of plants with friends as well as enemies.

## **In vitro Production of Strigolactones by Plant Root Cultures**

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Plant tissue culture, where aseptic conditions are maintained and cultural parameters are easily manipulated is a useful technique for investigating the biosynthetic pathways of target molecules. Preliminary screening of plant tissue cultures in our laboratory revealed that root cultures of *Menispermum dauricum*, *Stephania cepharantha* (Menispermaceae) and *Lotus japonicus* (Leguminosae) are copious producers of potent *Striga hermonthica* seed germination stimulants. EtOAc extracts of *Menispermum* and *Stephania* root culture filtrates induced considerable germination of *S. hermonthica* seeds, however, the hexane extract exhibited no activity. EtOAc and hexane extracts of *L. japonicus* root culture filtrate contained potent *S. hermonthica* germination stimulants. HPLC analysis of EtOAc extracts of *Menispermum* and *Stephania* root culture filtrates, each, showed one major fraction with germination-inducing activity, while that of *L. japonicus* root culture filtrate showed more than seven fractions with considerable activity. (+)-Strigol was isolated from EtOAc extracts of *M. dauricum* root culture filtrate. A major stimulant produced by *Stephania cepharantha* showed identical chromatographic behavior and molecular mass to authentic strigol. (+)-5-deoxystrigol was isolated from hexane extracts of *L. japonicus* roots extract and culture filtrate. The strigolactones were identified on basis of NMR, UV, MS and CD spectra and chromatographic behaviour on HPLC. Purification and identification of *S. hermonthica* germination stimulants from EtOAc extracts of *L. japonicus* roots and culture filtrate are currently under way. Authentic (+)-5-deoxystrigol was as active as GR24 in inducing germination of *Striga hermonthica* and *O. crenata* seeds. However, it was about 10- fold more active than GR24 in inducing *O. minor* seed germination.

## **The Arbuscular Mycorrhizal Symbiosis; Genomics Approaches to Dissect Development and Function**

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In natural ecosystems, most vascular flowering plants live in symbiosis with arbuscular mycorrhizal (AM) fungi. These mutually beneficial associations develop in the roots, where the fungus colonizes the cortex to obtain carbon from the plant. In addition to inhabiting the root, the fungus establishes hyphal networks in the soil, via which phosphorus and other mineral nutrients are transferred to the root. Thus, the symbiosis has a beneficial impact on plant health. Fossil evidence suggests that plants have been associated with AM fungi since they first colonized land and today, AM symbioses are found in ecosystems throughout the world. Development of the AM symbiosis involves signal exchange between the symbionts and this occurs prior to, and following, the physical interaction of the symbionts. Once inside the root, both the fungal hyphae and plant cells differentiate to create a specialized symbiotic interface, the arbuscule-cortical cell interface, over which nutrients are exchanged. Recent studies identified strigolactones as one of the pre-contact signals in the AM symbiosis indicating an overlap with plant-parasitic plant interactions<sup>1</sup>. It is possible that there are parallels in the later stages of the interactions also. We are using a legume, *Medicago truncatula* and an AM fungus, *Glomus versiforme*, to study the molecular events that underlie development and functioning of the AM symbiosis. By integrating transcriptional profiling information with reverse genetics approaches we have been able to identify plant genes whose expression is essential for the AM symbiosis.

1. Akiyama, et al. (2005) Nature 435: 824-827.

## **Calcium Mediated Transduction of Haustorial Inducing signals in *Striga asiatica***

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The sessile nature of plants has dictated a life plan in which development is tightly coupled to external signals in order to maximize survival. Such signals are then integrated through internal signaling pathways (i.e.  $\text{Ca}^{2+}$  and/or hormones) resulting in developmental changes. Deciphering the mechanisms of signal integration and subsequent organogenesis could have substantial agricultural benefits and may also shed some light on the developmental plasticity common to plants but largely absent in many other eukaryotes. In the parasitic angiosperm *Striga asiatica*, haustorium formation is initiated by the presence of host derived *p*-benzoquinones and is characterized by a halt in vegetative growth, swelling of the root tip, and the formation of haustorial root hairs. As both the initiating signal and the physical characteristics of the haustorium have been clearly defined for this parasite, *S. asiatica* represents an opportune system in which to explore the perception of host derived signals, or xenognosins, as well as pathogen development. From root hairs to pollen tubes directed growth, events are often mediated by elevated cytoplasmic calcium concentrations. Hypothesizing that both swelling and haustorial hair formation might be similarly regulated events we employed a variety of calcium chelators, channel inhibitors, and fluorescent probes to evaluate the importance of calcium in organogenesis. Our findings suggest that the perception of haustorial inducing xenognosin results in the rapid influx of calcium from an extracellular source, presumably the cell wall, which is crucial to further genetic and biochemical regulation. Furthermore, this work establishes rapid cytoplasmic calcium elevation, over a 15 minute period, upon introduction of the signal as the earliest component in the process of haustorial organogenesis observed to date.

## **Molecular Events During *Cuscuta* Infection**

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Dodder or *Cuscutaceae* are holoparasitic plants subsisting on other di- or monocotyledonous plant hosts. The infection process is initiated by adherence of *Cuscuta* prehaustoria to the host surface, followed by penetration attempts of hyphae. For a better understanding of these processes, some of the genes expressed in host tissue or in the parasite were isolated and the products characterized. For example, contact of *Cuscuta reflexa* prehaustoria to tomato induced expression of a plasma membrane bound cell wall localized Arabinogalactan protein (attAGP) in the host, right at the site of dodder attack. Using RNAi-technique and targeted virus induced gene silencing, a correlation of attAGP expression level and the parasite's attachment force to host tomatoes was observed. If the expression level of attAGP was reduced, *C. reflexa* attachment capability was also significantly reduced. *C. reflexa* infection induced a signal in the host leading to expression of tomato attAGP, which promotes the parasite's adherence. On the other hand, *Cuscuta* expresses a cysteine protease, which could be inhibited by an intrinsic repressor. Circumvention of cysteine protease activity during an active parasitic infestation by spraying an inhibitor solution on the plants, leads to reduced growth or death of *Cuscuta* and completely heals the former host plant. Since the inhibitor is a polypeptide, it is suggested to use it as a biodegradable, non toxic, *Cuscuta* specific pesticide.

### **Early Haustorium Development in *Triphysaria*: A View from Inside the Nucleus.**

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The competence to develop invasive haustoria is the defining feature of all parasitic species. We are identifying genes in the root parasite *Triphysaria* that are responsible for haustorium development in order to understand the evolutionary origin of this pathway in Orobanchaceae. In the longer term we are interested in learning the degree to which similar genetic pathways have been recruited in other parasitic families. Working under the assumption that genes associated with haustorium development will be differentially regulated soon after contact with a host root, we made cDNA libraries representing transcripts expressed in *Triphysaria* root tips after treatment with natural and chemical haustorial inducers. Nucleic acid hybridizations were performed to enrich the libraries for transcripts either up or down regulated after treatment with host roots, host root exudates, or purified haustorial inducing chemicals. These enriched libraries, as well as a library of cDNAs enriched for full length transcripts, were sequenced by the DOE Joint Genome Institute. Sequences of the ESTs and assembled unigenes are available as Fasta files at [http://www.plantsciences.ucdavis.edu/yoder/lab/Sequence\\_index.html](http://www.plantsciences.ucdavis.edu/yoder/lab/Sequence_index.html). Based on the distribution of transcripts in different libraries and on annotations of their putative functions, we selected a subset of genes for analysis by cDNA arrays. Candidate genes isolated on the basis of their expression profiles are being subcloned into RNAi vectors and introduced in *Triphysaria* roots by infection with *Agrobacterium rhizogenes* to monitor their functions *in planta*. Results and status of this work will be discussed.

**Stable Isotope and Nutrient Relationships of the Root Holoparasite *Hydnora* (Hydnoraceae) in Southern Africa**

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*Hydnora* is a genus of unusual plant parasites with a mainly African distribution. Most species of *Hydnora* reside underground and all lack stomata and leaves. Estimated transdermal water loss in *Hydnora africana* and *Hydnora triceps* rhizomes was 0.14<sub>-.02</sub> and 0.19<sub>-.02</sub> mg cm<sup>-1</sup> hr<sup>-1</sup>, respectively. Due to its extremely water conservative nature *Hydnora* may be a useful model for parasite-host uptake. We measured carbon and nitrogen natural abundance stable isotope ratios for 11 *Hydnora*-host associations in southern Africa, including host plants with dominantly CAM or C<sub>3</sub> metabolism. *Hydnora*-host isotope ratios were compared to 12 mistletoe-host associations, emphasizing relationships between host plants shared by *Hydnora* and mistletoes including *Tapinanthus* and *Viscum* species. Carbon and nitrogen isotope values for *Hydnora* are negatively correlated and carbon isotope ratios mirrored the host photosynthetic metabolism. For the first time we report mineral nutrition relationships for three holoparasite-CAM host associations. Total P and K levels were significantly elevated in the holoparasite relative to the host in most associations. Total Ca, Cl, Mg, N, Na, and S levels were significantly lower in the host.

## **Sap Flow from Host to Mistletoe: an Anatomical Approach**

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The mistletoe *Viscum album* L. is a hemiparasitic flowering plant. It is (partially) carbon autotrophic, but obtains water and nutrients by tapping into the wood of the host. Classical light microscopical methods and high-resolution 3D X-ray computed tomography were used on poplar (*Populus x canadensis* Moench, Salicaceae) infected with mistletoe (*Viscum album* L., Viscaceae) to understand water flow and development of the endophytic system. Direct vessel-vessel connections at the interface host-mistletoe ensure mass water and nutrient transport to the parasite. Almost every host vessel bordering the mistletoe haustorium had a simple perforation that directly connected them with the xylary elements of the parasite. The presence of the parasite showed a high and well directed influence not only on the quantity of wood produced by the host (hypertrophy), but also on the grain of the host wood. The mistletoe seems to direct actively the sap flow of the host towards its haustoria. In this respect, dimensions and morphology of the haustoria and host-mistletoe interface are of significant importance. The broad parasite vessels near the interface transport the sap to xylary elements with a smaller diameter, which conduct the water towards the cortical strands and the aerial parts of the mistletoe. Could this transition to narrower xylem elements pose a significant resistance for water conduction?

**The Sucrose-Degrading Enzymes in *Orobanche ramosa*. Characterization and Involvement in Growth, Cell Wall Synthesis and Starch Accumulation.**

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*O. ramosa* is an obligate parasite of major crops such as tobacco, tomato, oil seed rape and hemp. It has been known for more than two decades that the host-derived sucrose supports parasite growth. Nevertheless, sucrose metabolism in the parasite remains to be elucidated. We have initiated studies aiming at characterizing the molecular components, such as cell wall invertase (CWI), vacuolar invertase (VI), neutral/alkaline invertase (NI) and sucrose synthase (SuSy), involved in sucrose mobilization in *Orobanche ramosa* growing on tomato roots. Our first data show that tubercles display an equilibrated VI / NI ratio while this ratio is stronger in the growing subterranean stems due to a specific increase in VI activity. This reflects the key role of this enzyme during sucrose utilization in the growing stem where cell expansion occurs through vacuolar hexose and mannitol accumulation. The VI enzyme was purified using affinity and gel filtration chromatography. Only one isoenzyme was detected in stems, and the native form shows a pI of 3.8 and a molecular mass of 98 ± 7 kDa. Protein sequencing is in progress. CWI activity is constant to a basal level in tubercles and stems. In contrast, tubercles display a significant SuSy activity. Using antibodies raised against faba bean SuSy, we show that this enzyme is strongly associated to tracheids and amyloplasts contained in parenchyma cells. It suggests that in tubercles SuSy is involved in the sucrose mobilization required for cell wall polysaccharide synthesis in tracheids and for starch accumulation in parenchyma cells.

## **Deciphering the Interaction of Striga with Hosts and Non-hosts**

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The parasitic weeds *Striga gesnerioides* (witchweed) and *Alectra vogelii* are among the major constraints to the production of cowpea, *Vigna unguiculata* (L.) Walp., one of the most important food and forage legumes in the semi-arid tropics of West and Central Africa. There is considerable variation in host specificity among isolates of *S. gesnerioides*. Based on host differential response and genetic diversity analysis at least seven races of *S. gesnerioides* have been identified within the cowpea-growing regions of West Africa that specifically parasitize cowpea. Similar evidence also exists for multiple races of *A. vogelii* parasitic on cowpea. Race specific resistance genes have been identified and mapped into two linkage groups (LG1 and LG6) within the cowpea genetic map. How race specific resistance is mediated at the cellular and molecular level remains to be determined. Studies are underway aimed at identifying the factors that determine host specificity and virulence in parasitic angiosperms and to address whether specific avirulence genes exist in *S. gesnerioides* that correspond to the race-specific resistance genes identified in cowpea and if these form the basis for a gene-for gene interaction as recognized in other plant-plant pathogen interactions. Our findings indicated that developmental processes occurring post-germination and attachment are likely more important in limiting successful host parasite interaction. Progress in this work will be discussed.

## **The Molecular Basis of Susceptibility and Resistance to *Striga*: insights from Transcript Profiling**

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*Striga* species are among the most devastating parasites of subsistence farming agriculture in sub-Saharan Africa yet our knowledge of the mechanisms underlying host susceptibility and resistance lags behind that of fungal and bacterial pathogens. Understanding these processes at a molecular level is a critical step in the identification of genes that can be used for improving crop productivity via biotechnology based approaches, or via the development of molecular markers for use in marker assisted selection programmes. In a susceptible interaction, the *Striga* radical/haustorium attaches to the host root and the endophyte penetrates the epidermis, cortex and endodermis finally forming xylem continuity with the host. Once attached, both *S. hermonthica* and *S. asiatica* cause profound effects on the development and growth of the host plant. Recently we have examined changes in gene expression during a susceptible interaction between rice and *S. hermonthica* using Affymetrix oligonucleotide microarrays. Perhaps the most striking result was the extent of the down-regulation of gene expression that takes place as *Striga* develops on roots of the susceptible cultivar; of the 2588 genes that were differentially regulated, 553 were up regulated whereas over 2000 were down regulated. The down regulated genes included those purportedly involved in metabolism, cell cycle and DNA processing, transcription, protein synthesis and fate, cellular communication and signal transduction. Plants exhibit two different types of resistance; non host resistance where plants are resistance to all isolates of a particular pathogen species and host/race-specific resistance where they are resistant to some but not all isolates of a pathogen. In the latter, defense responses are triggered following the recognition by resistance gene (R-gene) products of specific pathogen or host derived elicitors. Whilst a large body of information is available concerning the molecular architecture of resistance to microbial pathogens very little is known about plant-plant resistance responses. In order to investigate the molecular basis of resistance to *S. hermonthica*, changes in gene expression were profiled in (1) the rice cultivar Nipponbare which exhibits host specific resistance and (2) *Arabidopsis thaliana* which is a non host to this parasite. In both cases over 1000 genes were significantly up or down regulated. The greatest number of genes was most strongly up regulated 24 hours post inoculation consistent with active host resistance responses. Functional analyses revealed that large numbers of genes involved in cell wall synthesis, defense signaling, regulation of transcription and protein synthesis, oxidative stress and primary and secondary metabolism were up-regulated but there were differences between the host and non host interaction. A key feature of the non host interaction was the up-regulation of many genes (EDS1, EDS5, PAD3, NPR1, NIMIN1, PR2) and WRKY transcription factors involved in the salicylic acid signaling pathway. Finally, susceptibility and resistance mechanisms will be considered in the context of physiological and metabolic interactions between host and parasite and a brief comparison drawn between plant-plant interactions and those involving interaction of plants with other taxa.

**Macromolecular Trafficking from Host Plants into the Hemiparasitic Plant *Triphysaria versicolor***

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Cross family transport of informational macromolecules such as mRNA, proteins and microRNAs and their significance in parasitism is poorly understood. Understanding how plants synthesize, transform and respond to these molecules will further our appreciation of host-parasite interactions and may lead to better strategies for vegetation management. However, a limitation has been the lack of transformation and assay systems for macromolecular trafficking in parasitic plants. We developed both *in vivo* and *in vitro* assay systems for studying macromolecule trafficking between the root parasite *Triphysaria versicolor* and its plant hosts. Transgenic roots were obtained on non-transgenic *Triphysaria* plants by *Agrobacterium rhizogenes* mediated *ex vitro* root transformation. Transgenic roots were also obtained from transformed calli. Micropropagated roots maintained their ability to make haustoria. Recent work showed that RNAi can traffic from a lettuce host to *in vitro* cultured *T. versicolor* roots. Transgenic GUSPlus *T. versicolor* root cultures were attached to lettuce generating double stranded RNA for GUS. Histochemical staining and semi-quantitative RT-PCR showed the silencing of GUS gene in root tips after haustoria connection with lettuce. In order to determine whether silencing signals are translocated throughout the parasitic plant, we made transgenic *Arabidopsis* bearing dsRNA targeting the *T. versicolor* phytoene desaturase gene (PDS). The hypothesis is that *Triphysaria* leaves will be bleached when *Triphysaria* is attached to *Arabidopsis* with the *T. versicolor* PDS construct, but not when attached non-transformed hosts. The experiments to determine whether siRNA mediated gene silencing of *T. versicolor* PDS occurs via haustoria connection are underway and will be reported.

## **Trafficking of Host mRNAs into Dodder: A New Frontier in Host-Parasite Communication**

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Dodders (*Cuscuta* spp.) have especially open connections to the host phloem and have been shown to obtain simple sugars, phloem-specific dyes, proteins, and viruses from their hosts. We have now shown that phloem-mobile mRNAs can also move from pumpkin (*Cucurbita maxima* Duch.) and tomato (*Lycopersicon esculentum* Mill.) hosts into attached lespedeza dodder (*Cuscuta pentagona* Engelm.). Tomato microarrays were used to probe RNA from dodder growing on tomato to reveal 474 putatively mobile transcripts. From this pool, over 50% of those tested have shown evidence of mobility based on sequence comparisons of reverse transcriptase PCR products generated from RNA of tomato, dodder grown on tomato, and dodder grown on other host species. To date, 21 tomato transcripts have been identified from dodder, including *GAI*, (GIBBERELIC ACID INSENSITIVE), which has been previously shown to be translocated in the phloem of tomato. Host-to-parasite movement of mRNA indicates a potentially new mechanism of interspecies communication, and raises many questions. For example, are the mobile host mRNAs used by the parasite to gain information about host physiological status? Does dodder distinguish host mobile mRNAs from its own, leading to selective accumulation or degradation of host mRNAs? Finally, the ability of dodder to form connections to taxonomically distant host plants simplifies identification of host mRNAs, so it is possible that dodder may be a useful tool for studying non-cell-autonomous RNAs in other plants.

## **A New Approach to Parasitic Weed Control Based on Silencing of a Key Metabolic Gene in the Parasite**

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The silencing approach has already been demonstrated as an effective control method against various pathogens. Gene silencing provides plants with defense against various pathogens, and is a tool of immense importance for research on plant development. The introduction of double-stranded RNA (dsRNA) proved to be a powerful tool for suppressing gene expression through a process known as post-transcriptional gene silencing in plants. In our study we used the inverted repeat technique for gene silencing of Mannose 6-phosphate reductase (M6PR), a key-gene in *Orobanche* spp. in order to provide the host plant with resistance against the parasite. A gene construct fusing the key gene for silencing to the binary vector (pBin-19) was already transformed to tobacco and tomato host plants. By PCR and RT-PCR analysis, transgenic plants were proved to have a specific PCR fragment (286 bp) which was designated on the mRNA of the *O. aegyptiaca* M6PR for silencing. Our results indicated that in-vitro production of small interfering RNAs (siRNAs) by introducing short double-stranded RNA molecules of the M6PR gene into *O. aegyptiaca* tubercles grown on tomato plants, facilitate suppression and degradation of the native M6PR mRNA, thereby reduction of total soluble solids (sugars) in the treated tubercles. Real-Time RT-PCR analysis showed that the endogenous M6PR mRNA of *O. aegyptiaca* tubercles or shoots grown on transgenic tomato plants harboring the M6PR silencing construct were reduced by 60-80% compared with the control (*O. aegyptiaca* grown on non-transgenic plants).

***Rhinanthus minor* as an Ecosystem Engineer: Understanding the Mechanistic Basis of Parasitic Plant-Induced Changes in Community Structure.**

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The root hemiparasite *Rhinanthus minor*, can suppress the abundance of grasses whilst promoting forbs in grassland communities, however, the outcome of its introduction is highly variable. We investigated the factors influencing this variability by studying the mechanisms through which the parasite acts to facilitate changes in community structure. Field manipulations confirmed that *R. minor*, suppresses the growth of grasses and whilst promoting that of forbs in the first growing season of a newly sown meadow. In contrast the removal of *R. minor* from mature meadow plots did not influence their floristic composition. In pot-based studies the parasite was highly damaging to grasses but had no significant effect on forbs although it did attach to them. Moreover, the parasite performed worst in terms of growth and photosynthesis when attached to forbs, even compared with unattached individuals. We hypothesized that forbs express resistant to the parasite. Tracer experiments using <sup>15</sup>N-nitrate supplied to the host confirmed this as the parasite abstracts more nutrients from grasses than from forbs. Histological studies established that all of the forbs possessed successful resistance mechanisms to haustorial penetration of host vascular tissues whilst no successful resistance was observed in the grasses. Two different resistance mechanisms were observed in the forbs; hypersensitive cell-death and host lignification at the host-parasite interface. Our results establish that differential host resistance underlies the community level effects of *R. minor* as forbs possess a resistance capacity that other potential hosts do not, consequently the parasite moderates intra-specific competition within grassland communities promoting forb abundance.

**Seedbank and Seedbank Management of *Orobanche ramosa* in South Australia.**

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The discovery of *Orobanche ramosa* L. in 1992 in South Australia has led to an intensive research and eradication effort. Seedbank longevity studies were initiated in 2002 and will be discussed, twice yearly exhumations are done but no significant reduction has been observed. Seed eradication with a soil drench "Bio Seed-Eradicator" containing novel, environmentally safe active ingredients has proved successful with up to 98% seed death. Trial results will be presented and discussed. Many herbicides and adjuvants have been tested with complete suppression of emergence from some products. Mainly conventional crop species have been involved but herbicide tolerant canola and herbicide tolerant Medicago have great potential due to a larger number of potent herbicides. Management leading to eradication will be discussed.

## **Size Dependent Parasitism of *Cuscuta gronovii*: Its Implications on Host Population Size Structure and Dynamics**

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Parasitic plants can have negative effects on the fitness of their hosts. However less is known of how parasitic plants affect the population properties of their host plants. Our research focuses on the genus *Cuscuta* (dodder), a holoparasitic plant that exhibits active host choice. We particularly examined: 1) the effects of parasitism by *Cuscuta gronovii* on mean population performance and size structure of its host *Pilea pumila*, 2) the mechanisms underlying active host choice in dodder, and 3) the relationship between size dependent selection and length of the parasitic association on the interactions of individuals within a population. The results of our experiments show that size dependent parasitism and compensation at the population level are the mechanisms underlying the increase of size inequality on parasitized plant populations. Dodder selectively establishes on the tallest individuals, suppresses their growth and continues to spread onto unparasitized plants that have become taller. Dodder is able to discriminate among potential hosts and choose the tallest ones within the population at least fifteen days prior to parasitism. The effects of dodder on host individuals are dependent on the length of parasitism; dodder causes a drastic reduction on vegetative and reproductive parameters on early attacked hosts compared to those attacked later. Under the studied scenario as well as in situations on which host plant size exhibits a positive correlation with competitive dominance, parasite foraging behavior as well as the responses of unparasitized individuals are crucial in understanding and predicting the outcome of parasitism in population plant properties.

## **Predictive Empirical Modeling of Parasitic Weed Life Cycle**

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Dormancy in *Orobanch*e and *Striga* seeds is removed as a two-stage process. First, after shedding from the mother plant, populations of dry *Orobanch*e seeds after-ripen (lose primary dormancy). Increasing the temperature results in a faster after-ripening rate of the seed population. The second stage – conditioning - occurs at water potentials close to full imbibition. The rate of conditioning in the seed population also increases with increase of temperature. Secondary or even tertiary processes may interfere with dormancy loss. For example, prolonging the conditioning period beyond about 21 days decreases the subsequent germination response due to a) induction of secondary dormancy and b) at temperatures over about 25°C, loss of viability in the case of *Orobanch*e. Germination of conditioned seeds depends on chemical stimulation from host's roots, temperature and water stress. The thermal time for germination is strongly affected by water potential. Non-linear probability models account for these processes and provide predictive empirical models to describe the germination behavior of the seed population. This paper discusses whether additive or multiplicative models are more appropriate for conditioning responses and explores the implications of these results for parasitism of crop plants.

## **Mechanisms of Resistance to Parasitic Plants: from Field Screenings to Laboratory Microscopic Studies**

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From about 4,000 species of parasitic angiosperms, only very few of them are weedy and parasitize cultivated plants. Among the main genera are *Orobanche*, *Striga*, and *Cuscuta*. The available control methods have not proven to be as effective, economical and applicable as desired. The only way to cope with the weedy root parasites is through an integrated approach, employing a variety of measures in a concerted manner. Genetic resistance remains as one of the most desirable components in an integrated control strategy. Because resistance is a multi-component event, understanding the physiological and genetic basis of such resistance at the different stages of the infection process will improve its utility as a control measure and assist in being attempts to integrate diverse mechanisms of resistance from different sources (including wild relatives) into a single cultivar (i.e., pyramiding multiple mechanisms of resistance). However, the basic mechanisms governing resistance to parasitic plants remain undiscovered. Furthermore, unlike the large core knowledge base available in other pathosystems, the molecular basis of the parasitic plant-resistant host interactions is still at a very preliminary stage. In this way, traditional histological methods are valuable tools in order to preliminarily unveil the defensive mechanisms leading to resistance. Recently, the combination of histological methods with molecular techniques (molecular histology) has been shown to be a powerful tool to increase our understanding and knowledge about the infection and resistance processes. We will try to give an overview about what is known on the mechanisms of resistance against root parasitic plants, mainly centred in broomrapes (*Orobanche* spp.).

## **Induction of Systemic Acquired Resistance in Root Parasitic Weeds**

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Induction of resistance was studied in red clover (*Trifolium pratense*) and rice (*Oryza sativa*) against root parasitic weeds by salicylic acid-mediated defenses. Experiments on the resistance induction in red clover against *O. minor* were carried out as follows. The seeds of red clover and *O. minor* were sown in pots packed with soil and incubated from the end of November in a greenhouse. Test chemicals were applied on the soil surface. Three months after incubation, the number of the tubercles was decreased by the treatment with BTH and tiadinil, inducers of SA-mediated defenses. Red clover seedlings were grown for 4 weeks in Petri dishes. The roots were treated with defense inducers for 3 hours. The seeds of *O. minor* treated with GR24 were sown on the roots of red clover. After a 5-week incubation, SA and BTH greatly reduced the number of the established parasites. The reduction was attributable to the development of haustoria before the radicle reached a host and the activation of defense responses in the host root including lignification of the endodermis. Experiment on resistance induction in rice against *Aeginetia indica* were carried out as follows. The seeds of rice (cv. Koshihikari) and *Aeginetia indica* were sown in pots packed with upland soil and tiadinil was applied to the soil surface. The pots were incubated in a greenhouse from the beginning of July. The shoots of *A. indica* emerged from the soil in late October in the non-treated pots. The number, height, and weight of *A. indica* shoots were decreased by the tiadinil treatment.

### **Molecular analysis of resistance mechanisms to *Orobanche cumana* in sunflower**

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In sunflower, resistance to *Orobanche cumana* is characterized by a low number of parasite attachments and a confinement of the parasite in host tissues leading to its necrosis. To help understand what are the mechanisms governing such resistance, a molecular approach was employed during both early and late responses of susceptible (2603) and resistant (LR1) sunflowers infected by *O. cumana*. The expression patterns of defense-related genes were investigated. The resistant genotype exhibited a stronger overall defense response against *O. cumana* than the susceptible one, involving preferentially marker genes of JA and SA pathways. Among them, *HaDef1* (defensin), appeared to be characteristic of the LR1 resistance. Plant defensins are small basic peptides of 5-10 kDa and most of them exhibit antifungal activity. In LR1, among the three defensin encoding cDNA identified, only *HaDef1* presented a preferential root expression pattern and was induced upon infection by the root parasitic plant *O. cumana* few days before necrosis occurred. A 5.8 kDa recombinant peptide, Ha-DEF1, was produced in *E. coli*. Investigation of in vitro antifungal activity of Ha-DEF1 showed a strong growth inhibition of *Saccharomyces cerevisiae* and a morphogenetic activity on *Alternaria brassicicola* germ tube development. Bioassays also revealed that Ha-DEF1 rapidly induced cell death at the radicle apex of *Orobanche* seedlings but not of another parasitic plant, *Striga hermonthica*, nor of *Arabidopsis thaliana*. These results demonstrate for the first time a lethal effect of defensins on plant cells. The putative mode of action of defensin in *Orobanche* cell death and its possible involvement in sunflower resistance will be discussed.

## **Striga Weed Management Options under Smallholder Agriculture in Africa**

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Agriculture is the main source of livelihood for most households in Africa. Damage caused to three most important crops, maize, sorghum and pearl millet by *Striga* spp. in Africa is devastating to resource-challenged farmers whose main source of livelihood can be threatened by complete crop loss to this root-parasitic weed. A range of effective component technology control practices focusing on factors such as hoe weeding and hand pulling; use of inorganic fertilizer and manure; crop rotations, fallowing, and early planting; use of *Striga* tolerant varieties; soil fertility management and herbicides have shown value in reducing losses - but these have been poorly adopted and have thus failed to slow the spread of *Striga*. New technologies to deal with the high levels of *Striga* in African soils must meet four criteria to be widely adopted by farmers: (1) have the ability to control *Striga* early in its growing cycle in order to reduce yield loss; (2) deplete the *Striga* seed bank in the soil; (3) be cost effective; and (4) be compatible with existing cropping systems and technologies. The most promising new *Striga* control practice in maize is coating seeds with resistance to ALS-inhibiting herbicides with herbicides such as imazapyr prior to planting. Extensive on-farm testing in several African countries has demonstrated the cost effectiveness of this technology. It is highly effective in reducing *Striga* incidence two-fold - in terms of reduced seed bank in the soil and decreased infection in maize. Integration with other control options and deployment of this technology offers medium-term solution for an urgent *Striga* problem in Africa until breeders identify sources of resistance for the long-term.

## **Temporal Thermal and Special Model for *Orobanche* Management**

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The overall goal of our study is to develop a decision support system for *Orobanche* control based on spatial and temporal sub-models. a) Temporal model: in previous studies, a mathematical relation between temperature and the parasitism process of *O. aegyptiaca*, *O. minor* and *O. cumana*, in tomato, red clover, and sunflower, respectively, was shown. A Growing Degree Days (GDD) model was developed to predict *Orobanche* parasitism in these crops. Using a sigmoid function allows us to predict the timing of the various *Orobanche* spp. developmental including subsurface stages. Herbicidal control of *Orobanche* was achieved in tomato, red clover, and sunflower using ALS inhibitors. The most effective control was achieved in the subsurface parasitism phase. Optimized chemical control is achieved when minimal herbicide rates are applied to the host at the most susceptible stage of the parasite. Herbicide application timing is based on the GDD model mentioned above. Recently, the option of *in-situ* monitoring sub-surface parasitism was introduced by using the minirhizotron camera. This is a non-destructive tool for detecting and monitoring parasitism over time. The minirhizotron technology is essential for verifying the herbicide rate models. This will allow making proper decisions regarding to herbicide application timing. b) Remote sensing approach was used for modeling the spatial distribution of *O. aegyptiaca* in tomato and in dill fields. IR, NIR and RGB aerial images were taken from satellite and from air for tomato and parsley fields infected with *O. aegyptiaca*. *Orobanche aegyptiaca* could be partially detected only by RGB aerial images.

## **Integrating Germination Patterns, Chemical, and Nonchemical Options to Manage Swamp Dodder in Massachusetts Cranberry Production**

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Current recommendations in Massachusetts cranberry production for the control of dodder, *Cuscuta gronovii*, include the use of horticultural practices (e.g., application of uniform sand layers) along with the application of preemergence herbicides. Multiple control strategies are needed to keep dodder from significantly reducing yields on an annual basis. A 2-year project was evaluated the efficacy of short-term floods (24 to 48 hr) for the control of dodder. In addition, using simulated bogs constructed in plastic containers, the germination patterns of MA dodder seed has been monitored for 9 years. Both studies should provide important information that will help growers improve the timing of management strategies. Grower trials and controlled research studies with flooding have documented variable effects that may be related to timing the flood to coincide with a certain period of seedling emergence. Dodder seeds apparently have a definitive peak of germination in the initial year after seeding. Peaks can still be seen in subsequent years, but they are much lower. Over the course of this 9-year study, the peak germination period occurred later each year. The delay of peak germination from populations in the seedbank causes overlapping generations to exist in a single farm field. This may contribute to the difficulties growers have in managing dodder. Since most herbicides targeting dodder can only be efficacious for a specific window of time, a portion of the population may always escape preemergence control and provide enough viable seedlings to cause substantial infestations in the vine canopy.

**Calculation of Growing Degree Days to Determine Optimum Timing of Herbicide Application for Control of Branched Broomrape *Orobanche ramosa* in Pastures.**

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In South Australia (SA), branched broomrape *Orobanche ramosa* is the subject of a national eradication program. Inspections, quarantine protocols and control treatments are in place for affected properties in the Murray Mallee region of SA to limit any further spread of branched broomrape and eliminate soil seedbanks in mapped infestations. At present the weakest link in the eradication program is the difficulty of controlling branched broomrape in pasture paddocks. The pastures in the area are generally unimproved and contain a range of host species of branched broomrape. Management of branched broomrape in pastures is difficult but can be achieved by spraying sulfonylurea or glyphosate herbicides onto weed hosts prior to broomrape emergence. The timing of herbicide sprays is important to target smaller attachments. Assessment of the timing of broomrape attachments and development until emergence was conducted in the field during the 2006 season. Additionally, observations were also collected on hosts grown in a glasshouse. The broomrape development data was correlated to growing degree days (GDD) using soil temperature and a base temperature of 0oC. Broomrape attachments began around 500 GDD after the start of the growing season, or after planting the hosts in the glasshouse. Emergence occurred 1000 GDD after attachment both in the glasshouse and in field conditions. Based on 2006 results, optimum herbicide application time would have been from 250 GDD after attachment to approximately 750 GDD after attachment. Further field and glasshouse observations are being conducted during 2007 to determine if the GDD model can accurately predict broomrape emergence in a different season and on different hosts.

## **Integrated *Striga hermonthica* Management Based *Fusarium***

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Cereal production is mortgaged by *Striga hermonthica* (Del.) Benth., the main angiosperm root parasitic. The pathogenicity of endogenous *Fusarium* isolates against *S. hermonthica* was evaluated in the laboratory and an application method was investigated under greenhouse conditions. Cultural control methods against the parasitic plant were also evaluated under field conditions in order to combine them with biological control using *Fusarium* in *Sorghum* field. The results of our study showed that the spores and lyophilized culture filtrate of some *Fusarium* isolates significantly reduce germination of *S. hermonthica* seeds. Furthermore, applying spores from *Fusarium* isolates to the seeds of *S. hermonthica* led to a potential reduction of the germination rate of *Striga* of more than 78% with eight *Fusarium* isolates. The effectiveness of the lyophilized culture filtrate to inhibit germination of *S. hermonthica* is related to the amount and type of isolate used in the experiment. Thus, the complete inhibition of germination is observed starting from 33 mg/ml with the lyophilisate of four *Fusarium* isolates and at 67 mg/ml with that of three others. This study showed the capacity of *Fusarium* spores to kill *S. hermonthica*. Application of spores of two *Fusarium* isolates on the plants of the parasite led to a reduction of more than 77% of *S. hermonthica* dry biomass. Two substrates were used to produce the inoculum-based *Fusarium*: compost and *Sorghum* chopped straws. Incorporating the inoculum into the soil to control *S. hermonthica* was effective. By using this method, six *Fusarium* isolates showed potential effectiveness in controlling *S. hermonthica*. However, their effectiveness depends on the growth medium. Burial of *S. hermonthica* seeds up to 10 cm from the sowing hill containing the inoculum did not influence the performance of *Fusarium* isolates to control *S. hermonthica*. On the other hand, burial depths of 10 cm below the sowing hill containing the inoculum influenced the effectiveness of *Fusarium* isolates. Irrespective of the substrate, the increase of the burial depth led to a reduction of the number of *Fusarium* isolates effective against *Striga*. The evaluation of the pathogenicity of *Fusarium* isolates in field revealed the possibility to integrate biological control to cultural control methods in the management of *S. hermonthica*. The incorporation of inoculum-based compost into the soil led to a great reduction of number of emerged *S. hermonthica* in plots. Integrated management of *S. hermonthica* combining use of *Fusarium*, a tolerant *Sorghum* variety, trap crops or organic/mineral fertilizer reduced more significantly infestation of *S. hermonthica* in the field and subsequently enhanced crop yield. Results from these experiments of different control methods against *S. hermonthica* enabled us to propose an integrated management approach of the parasitic plant. However, there is need for improved bioherbicide formulations and delivery systems to enhance the potential role of biocontrol in integrated management of the parasite in Burkina Faso.

## **Development of a Biological Control Strategy for Management of Hemlock Dwarf Mistletoe in Coastal British Columbia, Canada**

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The purpose of this research is to assess the indigenous fungi *Neonectria neomacrospora* (pathosystem 1) and *Colletotrichum gloeosporioides* (pathosystem 2) as biological control agents for the conifer parasite hemlock dwarf mistletoe (HDM; *Arceuthobium tsugense*). Two separate field trials were conducted on Vancouver Island. Treatments for pathosystem 1 involved spraying Stabileze formulated conidia onto unwounded and wounded HDM swellings and cut HDM shoots. Treatments for pathosystem 2 involved spraying Stabileze formulated conidia onto unwounded HDM swellings and cut HDM shoots, and spraying Sucrose-gelatin formulated conidia onto unwounded HDM swellings. The objectives of both field trials were to: (1) determine the efficacy of treatments in establishing infection, and (2) measure the impact of infection on HDM shoot health and number. Results for pathosystem 1 suggest that applying inoculum to wounded HDM swellings had a greater impact on *N. neomacrospora* infection than applying inoculum to unwounded HDM swellings or cut HDM shoots. HDM swellings with confirmed *N. neomacrospora* infection (isolation of the fungus and (or) presence of sporodochia) had their numbers of healthy mistletoe shoots significantly reduced by 1.6 or about 36% when compared with HDM swellings with unconfirmed infection ( $P=0.014$ ). Results for pathosystem 2 showed stabileze and sucrose-gelatin formulated conidia treatments reduced the current berry crop by 16-36%, respectively ( $P=0.05$ ). While the results for shoots appeared promising, heavy background infection and/or secondary infection, especially on controls, limited the ability to detect clear treatment effects. The implications of these results in variable retention silviculture systems and future research will be discussed.

## **Green manure: A *Striga* Management Technology Whose Time Has Come?**

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Soil fertility depletion contributes to low per capita food production and poverty in sub-Saharan Africa. An associated problem, often indicative soil fertility decline is increasing infestation of cereals by *Striga asiatica*. Farmer participatory research in Kyela District and Matambo Division of the Ulugulu Mountains in Tanzania has since 2000 identified low-cost approaches to increase agricultural productivity on *Striga asiatica* infested soils. Farmer groups in four villages in Kyela tested legume-cereal rotations and achieved average upland rice yield increases in 2003 and 2004 of 1114 (108%) and 827 kg ha<sup>-1</sup> (72%) respectively when planting after growing the green manure *Crotalaria ochroleuca* the previous year and an additional 119 kg ha<sup>-1</sup> (18%) when planting rice in 2004 after pigeon pea. Knowledge of the use of legume-rice rotations was disseminated to a further five communities and farmer-managed rice crops monitored in 2005 showed mean yield gains of 791 (74%) and 487 kg ha<sup>-1</sup> (46%) when planted after *Crotalaria* or pigeon pea compared to continuous rice. Across 16 sites in Matombo in 2005 mean maize yield was increased by 180 and 153% following *Crotalaria* and pigeon pea respectively. Green manures are not new and there is a history of limited adoption of soil fertility technologies in sub-Saharan Africa. Understanding how farmers see soil fertility challenges, involving them in the selection of “best bets” and identifying the knowledge that farmers need to adopt new methods successfully has been central to laying the foundations for up-scaling adoption of green manure on *Striga* infested land.

## PROGRAM OF POSTER PRESENTATIONS

### **Ecological and Physiological Investigations on *Orobanche* Species in the Spontaneous Flora of Romania**

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In contrast to the few weedy species most *Orobanche* species have remained wild flowers in the spontaneous flora. We are examining the occurrence and distribution of non-weedy *Orobanche* species in Romania, the soil properties and climatic conditions required for conditioning *Orobanche* seeds and the germination stimulants exuded by host plants (e.g., low or sufficient germination stimulants). The associated flora around each *Orobanche* spike was recorded and root exudates of the associated plants were tested for allelopathic effects (e.g., germination inhibition or inhibition of radicle exoenzymes required for penetrating into the host root). We are also testing possible reasons for low viable seed production or loss of seed by insects or fungi. The obtained results will be compared with the conditions in Baden-Württemberg, Southern Germany, where most of the Romanian *Orobanche* species occur also in limited populations. The results will contribute to understanding the ecosystems in which non-weedy *Orobanche* species grow. They may also provide information on the conditions under which *Orobanche* species may turn into weedy forms when ecological conditions change. Moreover, they should provide a basis for fostering rare *Orobanche* species in Botanical Gardens for the future. Methodological approaches and observations in Romania during the past two years will be presented.

## **Parasite Mediated Maternal Effects in Bitter and Sweet Lupins**

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A strong emphasis has been placed in the study of parasitic plants in forestry and agricultural settings given the potential for economic losses these “pests” have in their hosts via marked changes in growth, allometry and in many cases total crop failure. Most studies have overlooked the fact that parasitic plants can directly affect the growth environment of the maternal parent and thereby the development, size, viability and chemical composition of its progeny. Our research focuses on environmentally-mediated maternal effects of *Castilleja indivisa*, a hemiparasite, on high and low alkaloid content isogenic lines of *Lupinus albus*. We are particularly interested in examining the role and mechanisms by which the parasite affects host nutritional content and its implications in plant defense and reproduction. *Castilleja indivisa* is known to alter nutrient availability and allocation patterns of its hosts. *Castilleja* preferential uptake of N from its hosts is expected to result in a reduction in growth rate of host sinks (flowers and fruits) and seed provisioning (i.e. quality). Parasitized hosts are predicted to have lower seed size and greater seed C: N ratios than unparasitized hosts. However, since the intensity of parasitism is usually correlated with host N content, we predict a greater magnitude of maternal effects on high alkaloid hosts compare to low alkaloid hosts.

### ***Cuscuta* Species in Taiwan: Molecular Differentiation and Related Findings**

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*Cuscuta* in Taiwan consists of mainly three taxa: *C. japonica*, *C. japonica* var. *formosana*, and *C. campestris*. *C. japonica* and *C. japonica* var. *fomosana* parasitize woody hosts of lowland and mountain areas, respectively. *C. campestris* is predominantly associated with herbaceous hosts of lowland habitats. Additionally, *C. chinense* and *C. australis* have been documented, but the former is rare and the later has been noted as mis-identification of *C. campestris*. We used different PCR methods to study samples collected from widespread areas. ISSR (Inter Simple Repeat) markers could clearly differentiated morphologically diversified taxa of *Cuscuta*. ITS (Internal Transcribed Spacer) data revealed that *C. campestris* from different sites were relatively homogenous with similarity higher than 98%. However, ITS sequences among populations of *C. japonica* or *C. japonica* var. *formosana* were abnormally variable (similarity could be lower than 80%) for the species level. Our further investigations on ITS led to interesting findings on possible DNA transfer between *C. japonica* complex and their hosts.

**Genetic variability among five races of *Striga gesnerioides* (Willd.) Vatke detected by ISSR, AFLP and cpSSR analysis.**

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*Striga gesnerioides* (Willd.) Vatke is an obligate root parasite of several dicotyledonous species including cowpea (*Vigna unguiculata* (L.) Walp.), an important legume crop of the semi-arid regions of West Africa. Based on host-parasite interactions in the field, various races of *S. gesnerioides* attacking cowpea have been identified. In this study, we investigated the genetic variability within and between 44 populations of five of the previously recognized races of the parasite present in West Africa. Three different types of molecular markers were used on up to 10 individuals from each population. ISSR (inter-simple sequence repeat) markers showed no genetic variability within populations. The variability between the populations was also extremely low and did not allow discrimination of the five races. A few populations were more closely related (notably two populations from Togo), but no geographical or “racial” clustering could be seen. Further work with ISSR and AFLP (amplified fragment length polymorphism) markers is in progress to improve the accuracy of the analysis. Finally, we used cpSSR (chloroplastic simple sequence repeat) markers as these have been shown, in many studies, to reveal greater amounts of polymorphism at the intraspecific level. A total of 34 cpSSR primer pairs from various species were used, 26 of which produced amplicons in *Striga*. Absolutely no polymorphism was observed with cpSSR markers emphasizing that despite a large geographical distribution, very low genetic variability can be found in the different populations of *S. gesnerioides*. This may be due in part to the autogamous mode of reproduction of the parasite.

**Studies on Seed Dormancy, Germination and Seedling Emergence of *Striga Hermonthica***  
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This paper represents the first attempt to apply a conditioning model for parasitic weeds to seeds in soil in the glasshouse. The previous models developed for *Striga hermonthica* and for three *Orobanchae* species were only applied empirically and were not validated on independent data sets. The results indicate that *S. hermonthica* seeds recovered from eight soil environments of different drought and urea treatments, attained germination within 14-28 days and these optimum periods were similar to predictions based on the *in vitro* model, Once the seeds have been in imbibed storage in the soil for more than about 28 days in this experiment, induction of secondary dormancy increased but gradually, leading to a slow decrease in germination over a period of 91 days which in most environments did not terminate in zero germination. After 119 days of conditioning in the soil, germination percentages remain significantly high compared to the model predictions. The implication of the present findings for the control of *S. hermonthica* was discussed.

## Potential Use of Nijmegen-1 and Smoke Water Solutions to Deplete *Orobanche ramosa* Seed Banks in Greece

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Laboratory and field experiments were conducted to study the effect of using stimulants to deplete *Orobanche ramosa* seed banks. Germination stimulants tested in the laboratory were Nijmegen-1 (NE-1:  $10^{-4}$ ,  $10^{-6}$ ,  $10^{-8}$  and  $10^{-10}$  mol/L) and two smoke water (SW) solutions, ("Seed Starter" from Australia and a comparable solution produced by burning hay in Greece) were studied. SW concentrations tested were 100, 10, 1, 0.1 and 0.01% v/v aqueous solutions. In the field experiment, five treatments ( $10^{-5}$ ,  $10^{-6}$ ,  $10^{-8}$  mol/L of NE-1 respectively, 1% v/v local SW, and  $10^{-5}$  mol/L of NE-1+ 1% v/v local SW) were tested in a tobacco field. The field was initially uninfected with *Orobanche* and 500-1000 *O. ramosa* seeds were placed at marked locations to which tobacco plants were subsequently transplanted. The field site was kept moist for a week to allow the seeds to precondition after which the stimulant solutions were sprayed using a backpack sprayer at 93.5L/ha spray volume and 207 kPa of pressure. Plots were then kept moist for two weeks to allow seeds to germinate and die before transplanting tobacco to each location. Additionally, in each plot at least 100 seeds were placed in nylon mesh packets at 2.5 cm depth. These packets were exhumed and germination was recorded. The non-germinated seeds on exhumation were placed in Petri-dish (1wk, 23°C) with distilled water for further estimation of germinability of seeds. In the laboratory,  $10^{-6}$  mol/Litre NE-1 induced 38% germination as did the standard  $10^{-6}$  mol/Litre GR24. Lower and higher NE-1 concentrations exhibited much lower germination, at levels similar to water control (4% germination). The local SW stimulated 78% germination at 1% v/v concentration, higher than the "Seed Starter" SW (52%). At high concentrations, both SW strongly inhibited (less than 13%) germination. The "Seed Starter" SW at more diluted concentrations (0.1 and 0.01% v/v) exhibited a gradual decline of germination (45, 32% germination, respectively). In contrast, the local SW showed a 48% germination even at the lowest concentration (0.01%). Depletion of *O. ramosa* seeds, as recorded in the exhumed seed packets, was well over 50% with the optimum local SW treatment compared to 38% with  $10^{-6}$  mol/L NE-1. There was little difference between the other treatments (max. depletion 32%). Some caution should be exercised as germination was incomplete at the time of exhumation and was allowed to continue in petri dishes moistened with water. Germination of *O. ramosa* seeds *in situ* in the soil was  $\frac{1}{2}$  to  $\frac{1}{3}$  of the total seed germination. The use of smoke and smoke water to stimulate germination in both laboratory and field is well-known for a wide range of species although very little work has been carried out on parasitic weeds. The potential of smoke water to deplete the *Orobanche* soil seed bank is therefore interesting and the implications of smoke water and other stimulants such as NE-1 to deplete *O. ramosa* seed banks before transplanting susceptible crops will be discussed.

## **The Biosynthesis of the Tomato Germination Stimulants is Promoted by Phosphate Starvation**

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Plants produce a large variety of secondary metabolites to defend themselves against harmful organisms and to attract others that are beneficial. One class of these important signaling compounds are the strigolactones which are secreted by plant roots in extremely low concentrations<sup>1</sup>. Strigolactones constitute a group of secondary metabolites produced by plants to attract symbiotic arbuscular mycorrhizal (AM) fungi under nutrient deficient conditions, inducing hyphal branching<sup>2</sup>. However, at the same time they are used by the root parasitic broomrapes (*Orobancha* spp) and witchweeds (*Striga* spp) to germinate and complete their life cycle<sup>3,4</sup>.

Tomato (*Solanum lycopersicum* L.) is an important fruit crop in southern Europe, the Americas, the Middle East, and India, with increasing production in China, Japan, and Southeast Asia and their production is highly susceptible to infestation by *Orobancha ramosa* and *Orobancha aegyptiaca* that cause severe yield losses up to 75%<sup>5</sup>. We focus on the biosynthetic origin of the strigolactone germination stimulants for *O. ramosa* and hyphal branching factor for *Gigaspora rosea* by tomato cv. MoneyMaker plants. For this purpose both the inhibitor of carotenoids fluridone and tomato mutants were used and analyzed for their strigolactone production, and the pivotal role of these metabolites under nutrient deficient conditions will be discussed. In addition, we will show the progress of our research at the molecular level in the isolation and characterization of genes coding enzymes involved in the biosynthesis of the tomato strigolactones.

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## **Nitrogen and Phosphorus Deficiencies Promote the Production and Exudation of 5-Deoxystrigol in Sorghum**

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Strigolactones secreted from plant roots are important host recognition signals for root parasitic weeds and symbiotic arbuscular mycorrhizal (AM) fungi. In red clover, a host of *Orobanche*, a reduced supply of phosphorus, but not of nitrogen or other mineral nutrients, significantly promoted the exudation of orobanchol. By contrast, deficiency of nitrogen as well as phosphorus markedly promoted the exudation of 5-deoxystrigol from sorghum, a host of *Striga*. When sorghum plants were subject to deficiency of both nitrogen and phosphorus, the exudation of 5-deoxystrigol increased only slightly as compared to those under phosphorus deficiency. Sorghum plants continuously exuded 5-deoxystrigol under nitrogen deficiency, but the exudation was suppressed within 24 h after adding nitrogen to the growth media. Similar results were obtained with phosphorus treatments. Nitrogen and phosphorus deficiencies also increased 5-deoxystrigol contents in sorghum roots. In both cases the increase was comparable to that in the root exudates. These results suggest that strigolactones may be released rapidly after their production in the roots. Unlike the situation in the roots, nitrogen and phosphorus deficiencies did not increase the trace amounts of 5-deoxystrigol that are found in sorghum shoots.

## Identification of the Stimulants Produced by *Arabidopsis thaliana* Responsible for the Induction of *Orobanchae* Seed Germination

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The seeds of obligate root parasitic plants belonging to the *Orobanchaceae* require specific conditions for germination, including the unique requirement for induction by stimulants secreted by plant roots. In recent years a group of strigol-related natural stimulants from plant root exudates that induce root parasitic plant seed germination have been identified and termed as strigolactones. Strigolactones are abundant in plants from a wide range of botanical families, hosts and non-hosts of parasitic plants. In previous studies we have revealed that the model plant *Arabidopsis thaliana* induced germination and is susceptible to *Orobanchae aegyptiaca*, *Orobanchae ramosa* and *Orobanchae minor*. The aim of the present study was to produce, extract, purify and analyze the strigolactone seed germination stimulants exuded by the roots of wild type *A. thaliana* that are responsible for the induction of *Orobanchae* spp. seed germination. To produce *A. thaliana* root exudates, a novel hydroponics culture system was developed and exudates were collected on activated charcoal placed in the culture growth medium. Exudates were extracted from the activated charcoal with ethyl acetate and concentrated *in vacuo*. The crude extracts were analyzed using high performance liquid chromatography connected to tandem mass spectrometry. All chemical analyses were coupled with *Orobanchae* spp. seed germination bioassays. The root exudates chemical analyses and accompanying bioassays revealed several strigolactones that are active in *Orobanchae* spp. seed germination. Orobanchol was identified as a major *Orobanchae* spp. seed germination inducer produced by *A. thaliana*. The final identification of other detected known and novel strigolactones is in progress.

### **Trehalose Promotes Seed Germination of a Holoparasitic Plant, *Orobanche minor* Sm.**

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Parasitic plants greatly damage crops mainly in the semiarid Mediterranean regions. The establishment of technology to prevent their reproduction is eagerly required. It is well known that germination stimulants are required for the germination of the parasites. The seeds have to imbibe water at suitable temperature in the dark for several days to have the potential to accept the germination stimulants. This process is called conditioning. In this paper, metabolic profile of hydrophilic primary metabolites in seeds of *Orobanche minor* Sm. during the conditioning period was analyzed by GC/MS. As a result, 25 metabolites, such as organic acids, amino acids and sugars, were identified. Among them, the amount of trehalose significantly increased during conditioning. So we investigated the effect of exogenous trehalose on the germination. When 0.1 to 1% of trehalose was supplied during conditioning, the germination rates were increased 15% to 20%. This effect was also observed with germination inhibitors such as abscisic acid and glucose. Trehalose is hydrolyzed to two glucose molecules by trehalase in plants. Validamycin A, which is an inhibitor of trehalase, was shown to increase the endogenous trehalose and consequently the germination rates of *O. minor*, when it was supplied during conditioning period. From these results, we concluded that trehalose is synthesized during conditioning and has positive effect on the seed germination of *O. minor*. This information might be helpful to establishment of an efficient system of suicidal germination to prevent parasitic weeds.

**Yield increase in oat-faba bean intercrops under heavy *Orobanche crenata* infections.**

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*Orobanche crenata* (crenata broomrape) causes severe damage on legumes. Several cultural, biological, and chemical strategies have been suggested but none is completely successful or practicable in low input farming systems. We showed previously that broomrape infection on legumes is reduced in intercrops with oat. In the present experiment we studied the effect of broomrape reduction due to oat-faba bean intercrops on faba bean and broomrape biological dry matter (BDM). The assay was conducted in an infested experimental field in Córdoba, Spain in organic conditions. Susceptible faba bean cv. "Prothabon" was grown as sole crop and mixed with oat cv. "Cory" in 50% replacement model intercrop. Crops were laid out on plots (1.5x8 m<sup>2</sup>) in a complete randomized block design with three replicates. The high Land Equivalent Ratio value (LER=1.3) achieved shows that environmental sources for oat-faba bean growth are used on average 30% more efficiently in the intercrops than in the respective sole crops due to a) the degree of resource complementarity of faba bean-oat system in absence of any fertilization and b) the reduction of crenata broomrape attached per faba bean plant due to the inhibitory effect of oat. Faba bean BDM was 928 g/m<sup>2</sup> in sole crop and 666 g/m<sup>2</sup> in intercrop. Broomrape BDM was reduced from 193 g/m<sup>2</sup> to 42 g/m<sup>2</sup> due to intercrop, reducing proportionally the number of seeds laid in soil.

### **Yield losses in pea as a function of *Orobanche crenata* levels of infection.**

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Reduction of biomass and seed yield and size were studied on pea (*Pisum sativum* L.) as a function of level of infection by crenata broomrape (*Orobanche crenata* Forsk). The susceptible pea cultivar "Messire" was sown in a distance between plants and rows of 0.5 meter in a highly but not homogeneously naturally infested plot, what allowed us to study yield components in pea plants with a variable level of infection, ranging from 0 to 21 emerged broomrapes per pea plant. Pea productivity was measured as dry total biomass ( $DM_p$ ), seed yield ( $SY_p$ ) and number of seeds ( $NS_p$ ) measured per pea plant. The infection level was studied per pea plant determining the number of emerged broomrapes ( $N_b$ ) and parasitic dry matter ( $DM_b$ ) measured at harvest. The following models were used for the prediction of productivity losses. Dry total biomass of pea was reduced with infection, as a function of number of emerged broomrapes  $DM_p = 8.66 + 15.04e^{-0.20 \cdot N_b}$  ( $r^2=0.64$ ), or broomrape biomass,  $DM_p = 8.38 + 16.23e^{-0.30 \cdot DM_b}$  ( $r^2=0.63$ ). Pea seed yield were influenced by the infection level, both by number of broomrapes  $SY_p = -3.96 + (103.86 / (6.14 + N_b))$  ( $r^2=0.73$ ) and broomrape biomass,  $SY_p = -3.20 + 13.74e^{-0.12 \cdot DM_p}$  ( $r^2=0.67$ ). This reduced yield was due to a decrease of number of seeds per pea plant  $NS_p = -2.30 + 54.98e^{-0.14 \cdot N_p}$  ( $r^2=0.70$ ) and  $NS_p = -17.49 + 70.30e^{-0.11 \cdot DM_b}$  ( $r^2=0.66$ ), whereas seed weight was not influenced by infection level.

**Histochemical analysis of defense responses involved in resistance of sunflower (*Helianthus annuus*) to *Orobancha cumana***

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Sunflower broomrape (*Orobancha cumana* Wallr.) is considered as one of the major constraints for sunflower production in Mediterranean areas<sup>1</sup>. Breeding for resistance is regarded as the most effective and environmental friendly solution to control this parasite. However, the existing sources of genetic resistance are defeated by emergence of new more virulent races of the parasite.

In this work we have analysed the interaction between sunflower and *O. cumana* in order to get insights into the mechanisms involved in resistance. The interaction between *O. cumana* and two sunflower genotypes showing different behavior against the race F of *O. cumana*, HE-39998 (susceptible) and HE-39999 (resistant), were monitored by pot and Petri dish bioassays. Then, using histochemical procedures and microscopic observations, compatible and incompatible interactions were compared and several defense responses involved in resistance were identified<sup>2</sup>. Suberization and protein cross-linking at the cell wall were observed in the resistant sunflower cells in contact with the parasite, preventing parasite penetration and connection to the host vascular system. In addition, fluorescence and confocal laser microscopy observations revealed accumulation of phenolic compounds during the incompatible interaction, which is in agreement with these metabolites playing a defensive role during *H. annuus* - *O. cumana* interaction<sup>3</sup>.

1. Shindrova, et al. (1998) *Helia* 21: 55.
2. Echevarría-Zomeño, et al. (2006) *Journal of Experimental Botany*, 57: 4189.
3. Serghini, et al. (2001) *Journal of Experimental Botany* 52: 2227.

## **Response of *Medicago truncatula* Accessions to Various Species of *Orobanche***

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There is increasing interest in the legume species *Medicago truncatula* as a model in genomic studies. Genotypic variation for the mechanisms of resistance was found between *M. truncatula* accessions with an accession highly resistant and another highly susceptible to *O. aegyptiaca*, *O. foetida* var *broteri* and *O. ramosa*. Similar variation, but in reverse, was observed with *O. nana*. *In vitro* studies showed that *M. truncatula* can induce high levels of *O. aegyptiaca*, *O. foetida* var *broteri*, *O. nana*, *O. minor* and *O. ramosa* seed germination, but rather low levels of *O. cumana*, *O. crinita* and *O. crenata*. Differences in germination level among accessions were highly significant. Accessions also significantly varied in the penetration response to *O. aegyptiaca*, *O. foetida* var *broteri*, *O. ramosa* and *O. nana*. Increasing the germination level on *O. cumana*, *O. aegyptiaca*, *O. ramosa* and *O. minor* through applications of the synthetic germination stimulant GR24 did not result in increased infection. However, infection by *O. nana* increased markedly in the resistant accession. GR24 was not effective in inducing germinating *O. crinita*, *O. foetida* var *broteri* and *O. foetida* var *foetida* seeds. The variation observed for induction of germination of these species by *M. truncatula* and of subsequent attachment will be useful to isolating and characterising genes involved in early stages of *Orobanche* - host plant interaction and for the study of the biosynthetic pathways of production for germination stimulants.

## **Laser Capture Microdissection (LCM): New Technologies Apply to Study of the Parasitic Plant Interactions**

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Using Laser Capture Microdissection (LCM) technique, individual cells can be isolated from tissue sections for the profiling of gene expression. For these reason, we have chosen this method for RNA extraction of cells from tissues of *M. truncatula* infected with *O. ramosa*, an important parasitic plant pest of legumes crops. We selected *M. truncatula* as a model plant due to its similarities with other legumes which have complex genomes to analyze. Then we could analyze the infection process and the defense mechanisms against the attack of this pathogen. Basically, the slide with tissue of interest is placed under an inverted microscope and the image is transferred to a computer screen. A film-coated cap is placed onto the tissue. We select cells of interest and an infrared laser is pulsed at the selected region and heat generated determines the melting of the thermoplastic film from the cap with the tissue in correspondence to the demarcated region. Finally, the target region is selectively pulled away from the surrounding tissues when the cap is removed and samples are collected in a tube, now ready for molecular analysis. Other authors have analyzed this interaction using the whole tissues with the consequence being dilution of their RNA of interest in the total plant RNA. However, with this technique we attempted to analyze the transcriptome of target cells involved in the pathogenic process because we selected only the RNA of cell responding in this interaction.

## **Differential expression proteomics to investigate responses and resistance to *Orobanche crenata* in legumes**

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In order to study the plant response to parasitic plants and the molecular basis of the resistance we have used a proteomic approach. The root proteome of two accessions of the model legume *Medicago truncatula* and crop pea displaying differences in their resistance phenotype, in control as well as in infected plants have been compared. We report quantitative and qualitative differences in the two-dimensional gel electrophoresis (2-DE) maps of different plant genotypes and plant subjected to different treatments (control, non-infected, and infected). Differential spots were successfully identified by peptide mass fingerprinting (PMF) following MALDI-TOF mass spectrometry. Many of the proteins identified that show significant differences between genotypes and after parasitic infection belong to the functional category of photosynthesis and energetic metabolism, and defense and stress-related proteins. Data will be presented and discussed in terms of plant spp. and genotype common and differential responses, as well as compared with those previously obtained by using classical biochemical and transcriptomic approaches. Data have been published or submitted (Castillejo et al., 2004. *Phytochemistry* 65: 1817; Jorrin et al., 2006. *Euphytica* 147: 37; Rossignol et al., 2006. *Proteomics* 6: 5529; Rispaill et al., 2007. *New Phytologist* 173: 703; Castillejo et al., 2007. *Plant Physiology* (submitted).

## **Analyse of *Medicago truncatula* Resistance Against *Orobanche crenata* Using Citochemical Techniques**

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*Medicago truncatula* has emerged as an important model plant species for structural and functional genomics. The close phylogenetic relationship of *M. truncatula* with crop legumes (like faba bean, pea, vetches and lentil) increases its value as a resource for understanding resistance against *Orobanche* spp. *Orobanche crenata* is a root parasitic weed that represents a major constraint for grain legume production in Mediterranean and West Asian countries. Different cytological and histochemical methods (like stains, light and fluorescence microscopy, confocal studies, etc.) were used to study the mechanisms of resistance against *O. crenata*. For this study we have chosen two accessions of *M. truncatula*, showing early and late acting resistance. In the early resistance accession (Mt196) we found that the parasite died before a tubercle had formed. In the late resistance accession (Mt53) the parasite became attached without apparent problems to the host roots but most of the established tubercles turned dark and died before emergence.

**Biological Control of *Orobanche cernua* Seed Germination Utilizing an Indigenous Actinomycete Isolate in Jordan**

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An indigenous *Streptomyces* isolate, R9, was recognized to have phytotoxic activity against seed germination of weeds. It was also evaluated for its potential activity against *Orobanche cernua* seed germination under laboratory conditions. Cell-free culture filtrate (1 liter) of R9 (grown on glucose peptone molasses (GPM) in shaken broth culture for 7 days at 28°C) was lyophilized. Sterile distilled water and GPM broth were used as controls. Preconditioned *O. cernua* seeds were uniformly spread on the surface of water agar plates and irrigated with 1 ml of either lentil *Lens esculenta* root exudates or GR-24 (0.2 µg/l) as germination stimulus several hours before treatment. R9 extracts (250 µl) were transferred into wells (10 mm in diameter) cut in the center of each plate. Plates were incubated in a humid incubator at 25°C for 3 days, then percent seed germination was calculated around wells using a dissecting microscope at 45 X. Results showed 0% *O. cernua* seed germination for the R9 culture filtrates in contrast to 31.3% to 50% and 2.3% to 8% seed germination in the water and GMP controls, respectively, in duplicate experiments. Hence, R9 may be considered as a potential source for bio-herbicides to control *Orobanche*.

## **Integrated Management of Broomrape in India**

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Bidi tobacco is a non-Virginia tobacco, being cultivated in Nipani (India) for the 45 years under rainfed conditions. In India, *Orobanche cernua* is the most pernicious parasitic weed in tobacco cultivation. The severity of broomrape problem is increasing because of mono-cropping of tobacco and hand weeding is the only practice to control it. Among different chemicals tested GR 24 induced 58% germination of broomrape seeds under laboratory conditions. The positive interaction between GR 24 (1.0 ppm) and the root exudates of few crops deserves further attention. In trap crop field experiment, sunhemp and greengram crops reduced broomrape population by 68% and increased tobacco yields by 45% at 90 days after transplanting (DAT). Glyphosate and Imazaquin at 0.50 kg ai/ha and 0.01 kg ai/ha, respectively, reduced the number and dry weight of broomrape spikes. However, we suggest the following integrated cultural and chemical control approaches to obtain higher tobacco yields and minimize broomrape population in the soil for tobacco areas in Nipani and areas of similar conditions in India: (1) Grow trap crops (sunhemp/greengram) in the early spring and incorporate *in situ* at 45 days after sowing; (2) Transplant tobacco after 15-20 days; (3) Take up general hand weeding within 45 DAT; (4) Apply glyphosate at 60 DAT at 0.50 kg at ai/ha (or less); (5) Remove the remaining few broomrapes spikes by hands or apply plant oils to prevent seed formation. Also a cropping system with trap crop - short fallow - tobacco is an ideal practice to reduce the broomrape seed inoculum's potential in the soil.

**Potential of Establishing Root Hemiparasitic Sandalwood (*Santalum album* L.) as a NTF Species in the Buffer Zones of Forests and Degraded Lands in Australasia: a Sri Lankan Experience**

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*Santalum album* has received much attention during the recent years in Australasia due to its potential as a valuable NTF species. Sandalwood oil is used to prepare expensive perfumes, cosmetics and a range of medicines. One kg of Sandalwood oil is currently traded around US\$ 3,000. The potential exists to reduce harvesting pressure on natural stands of *S. album* by establishing plantations. Around 80% germination was obtained when seeds were treated with 750 ppm gibberellic acid. When the seedlings were grown in equal parts of sand, soil and farm yard manure, a significantly greater ( $p < 0.001$ ) height, root collar diameter and leaf number were obtained. A significantly higher ( $p < 0.01$ ) performance of sandalwood seedlings, with respect to light, was observed between 25% - 50% ( $1300 \text{ - } 915 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ ) shade and with respect to the pot hosts, with *Mimosa pudica* and *Tithonia diversifolia*. The best growth in terms of plant height, root collar diameter, root biomass, over a 3 year field establishment trial was obtained in the treatment where a sandalwood plant was surrounded by four *Sesbania grandiflora* plants at a spacing of 1.5 m apart from each other. Field studies showed the potential of a range of native host plants that could be intercropped with Sandalwood. This improves the enhancement of natural biodiversity, creation of new habitats and conservation of plant species in degraded areas. All this would not have occurred with out a commercial incentive to growers, which in this case is Sandalwood.

**Germination and Viability of *Cuscuta* spp. (Dodder) Seeds after Digestion in Sheep Rumen**  
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Studies were conducted to examine the effect of sheep rumen digestion on germination and viability of *Cuscuta* spp. seeds in comparison to non-parasitic weed seeds and two crops (barley and lentil). Seed samples were put in small monofilament nylon bags and placed in the plastic rumen of fistulated sheep for 1, 2, 3 and 4 days, respectively. Total percent germination (Germination + viability) of seeds four days after placement (DAP) in the rumen were 85% for *Cuscuta* spp., 31% for *Convolvulus arvensis* and 17% for *Chenopodium album*. Seeds of *Lolium multiflorum* and *Raphanus raphanistrum* died three DAP, while seeds of lentil and barley died one DAP. The results indicate that grazing sheep could be an important vector for carrying viable weed seeds, particularly *Cuscuta* spp. seeds, in their digestive system and depositing them with their feces in un-infested fields.

**Varietal Differences in Ability of Sesame and Pigeon pea as Trap Crops to Induce Suicidal Seed Germination in *Striga hermonthica* (Scrophulariaceae)**

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*Striga hermonthica* is the most serious constraint to maize production in West Africa Savannah. Seventeen (17) varieties of sesame and 13 accessions of pigeon pea were evaluated for their ability to stimulate *Striga* seed germination *in vitro* using cut-root technique. The test crop varieties evaluated showed significant differences in their ability to stimulate seed germination in *S. hermonthica*. On the basis of the results, the trap crops varieties were grouped into five with respect to stimulant production, viz., very high, high, moderate, low and very low. For Sesame: Very high (none), high (69B and Yandev \_ 55) moderate (Ex-Pankshin-98, 73A-79B, Cross-95, E-8, Ncriben-01M, 73A-H2B, 60-2-3-1-8B and Ciano-16), Low (Yandev-75, Type-4, Ciano \_ 27, Eva and 69-1-1) and very low (Pachequeno and Ncriben-03L). For Pigeon pea: very high (TCC1035, Cita 4, TCC6, TCC8126, Cita 3, TCC 87 and Cita 2), high (TCC2, TCC 8127, TCC 151, and Cita 1), and moderate (TCC8 and TCC 8129). The use of trap crops, which stimulate suicidal seed germination in *Striga* fits into the cultural practices of the small - scale farmer in Nigeria. The traps crops used in this study are cash crops and commonly grown in Benue State - Nigeria. However, varietal differences exist between and within trap crops. The varieties of sesame and pigeon pea belonging to the very high and high stimulants production groups are therefore recommended for use by farmers in Nigeria to clean up or reduce the reserve of *Striga* seeds in the soil.

**Photoresponse Analysis of Phytochrome A in the Non-photosynthetic Parasitic Plant;  
*Orobanche minor* Sm.**

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Among environmental factors, light induces various morphological responses. Photoreceptors are involved in these responses. Among photoreceptors, phytochrome is the major red and far-red light photoreceptor. After light perception, phytochromes localize from the cytoplasm to the nucleus and control the expression of downstream genes. The entire network of light signaling has not yet been elucidated because of its complexity. Therefore, we studied phyA of non-photosynthetic parasitic plant, *Orobanche minor*. This plant cannot photosynthesize and acquires its energy from the host plant. It is thought that *Orobanche minor* has the light signaling system related to photosynthesis partially altered, but it retained the function necessary to regulate morphogenesis. The amino acid sequence of *OmphyA* was compared to that of *AtphyA* from *Arabidopsis thaliana*. The result revealed that *OmphyA* has 26 amino acid substitutions and 71% sequence identity. These substitutions are largely located around functional domains. Therefore, we compared the physiological responses of *O. minor* with photosynthetic plants to find functional differences. As a result, *OmphyA* has the same biological characteristics as its counterparts in green plants. For the photoresponses, *OmphyA* has the same role as the green plant in germination and accumulation of anthocyanin. *OmphyA* plays an opposite role in inhibition of the stem elongation. By the usage of *A. thaliana*, it was revealed that the expression level of genes involved in phyA signal network was different in *OmphyA* and *AtphyA* transfected protoplasts. These results suggest that *OmphyA* function is different from that of *AtphyA*, and these differences may attribute to amino acid substitutions of *OmphyA*.

**Characterization of the Tocochromanol Content and Composition of Different Species of the Parasitic Flowering Plant Genus *Cuscuta***

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The holoparasitic plant genus *Cuscuta* comprises plant species with different degrees of plastid functionality, ranging from intact photosynthetically active chloroplasts, via plastids with impaired protein production and gene expression to plastids with reduced plastome gene content. While some species are photosynthetically active and have a chlorophyll and carotenoid composition similar to non-parasitic plants, other species are non-photosynthetic and do not contain any chlorophyll. To investigate whether the tocochromanol content and composition is related to the photosynthetic capacity of the species, the overall content of tocochromanols and the levels of the individual tocochromanols were determined in stem sections of eight *Cuscuta* species. All species including the non-photosynthetic ones contain plastoquinone and tocochromanols at variable levels. In addition, unusual tocochromanol species were detected in *C. japonica* and *C. grandiflora*. No correlation between tocopherol content and carotenoid content or chlorophyll content was observed indicating a function of the tocopherols unrelated to antioxidative tasks.

**Structural and Functional Attributes of the Hypogeous Root Holoparasite *Hydnora triceps* Drege & Meyer (Hydnoraceae)**

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Achlorophyllous root holoparasites with extremely reduced vegetative bodies, *Hydnora* spp. are the only known angiosperms lacking leaves and scales. *Hydnora* occur in the arid regions of Africa and the southern portion of the Arabian peninsula. Five species are currently recognized in this genus. The most remarkable species, *H. triceps* completes its entire lifecycle below ground. The reduced vegetative structure of *H. triceps* raises two important questions: (i) How does the vegetative body grow and attain its distinctive structural entity? and (ii) By what means does it make intimate connections with its host and fulfill water and nutrient requirements? The vegetative body consists of “pilot roots”, poorly studied structures bearing haustoria and flowers. Our studies showed a “chimeral” apical structure and a protective layer of cells similar to a root cap at the growing tip. However, eustelic, endarch and collateral vascular bundles and the continuous pith indicate that pilot roots are stems. Increased girth is due to the fascicular cambium, periderm and strands of cells distributed in the main vegetative body similar to a primary thickening meristem. The haustorium and the reproductive structures of *H. triceps* initiate exogenously from the “root bumps” (outgrowths of the vegetative body). An endophytic primodium penetrated the host root with a stratified meristem giving rise to xylary and phloic hyphae, and transfer cells.

### **Dodder (*Cuscuta pentagona*) Control in Roundup Ready Alfalfa**

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Dodder (*Cuscuta pentagona* and *C. indecora*) commonly infests alfalfa, reducing yield but also making it hard to dry after cutting. In greenhouse experiments, dodder (*C. pentagona*) was allowed to attach to Roundup Ready (RR) alfalfa, and treated with glyphosate at 0.84 or 1.68 kg ae/ha when dodder stems were 15 to 20 cm in length. Neither rate controlled dodder and dodder growth resumed within a week after treatment. Increasing glyphosate rate to 3.36 kg ae/ha or sequential treatments of 1.68 kg ae/ha made at two week intervals, resulted in 99% control. In a field study, dodder (*C. pentagona*) was allowed to attach to RR alfalfa. Glyphosate (0.84 or 1.68 kg ae/ha) or imazethapyr (0.10 kg ai/ha) treatments were made when the attached dodder stems reached 10 to 20 cm in length, with subsequent treatments applied two weeks later. Dodder control with glyphosate treatments was greater than 90% regardless of rate, while imazethapyr treatments provided less than 50% control. Imazethapyr treatment stunted the dodder, but dodder growth resumed within a few weeks. Following alfalfa harvest, dodder was observed to be completely controlled on all glyphosate plots except those that received a single application of the low rate (0.84 kg ae/ha). Although glyphosate effectively controlled dodder in the field study, further field testing is being conducted to verify this result, since dodder easily survived glyphosate treatment in greenhouse studies.

**Genetic Diversity Among *Orobanche crenata* Ecotypes Revealed by RAPD and AFLPs Markers, in Algeria**

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The objectives of this study were to investigate the efficiency of RAPD and AFLP marker systems in detecting intraspecific genetic polymorphism, and to determine the level of diversity among six *O. crenata* ecotypes, from different geographical localities of El Mitidja. Ten decamer RAPD primers and, three AFLP primer combinations (EcRI/MseI), generated a total of 144 and 429 markers respectively. Dice's similarity matrices were prepared and used for analysis. The diversity index, effective multiplex ratio, and marker index values were higher for the AFLP. This technique appears to be more useful than RAPD in the analysis of limited genetic diversity among the 6 ecotypes of *O. crenata* tested. Both marker systems were able to discriminate among materials analyzed, but clear distinction between all ecotypes was obtained with AFLP markers. AFLP is therefore another tool for assessing genetic relationships among *O. crenata* ecotypes along with RAPD. The study, showed a proportional increase in genetic distance with geographical distance, when going from the center of El Mitidja to the eastern-center and the western-center respectively. It is probable that the dissemination center of this parasitic plant is the center of El Mitidja.

## **The Strigolactone Germination Stimulants of the Plant-Parasitic *Striga* and *Orobanche* spp are Derived from the Carotenoid Pathway**

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The seeds of the root-parasitic *Striga* and *Orobanche* spp are only able to germinate after induction by a chemical compound(s) exuded from the roots of their host. For both *Orobanche* and *Striga* spp. several germination stimulants were identified from host and non-host plants. In most cases, the compounds were shown to be isoprenoid and belong to one chemical class, collectively called the strigolactones. Strigolactones have been detected in many plant species showing that they are wide-spread in the plant kingdom. Because the germination stimulants are produced/exuded in extremely low concentrations, an analytical approach to discover the biosynthetic origin of the germination stimulants is difficult. Therefore we used the high sensitivity of parasitic plant seeds to the germination stimulants and used a germination bioassay-guided approach to unravel the biosynthetic pathway. To assess the involvement of the isoprenoid biosynthetic pathways we investigated the induction of *Striga hermonthica* seed germination by root exudates of maize seedlings treated with inhibitors and of a series of maize mutants. We also used these inhibitors on cowpea (*Vigna unguiculata*) and sorghum (*Sorghum bicolor*) and assessed the effects on the root exudate-induced germination of *S. hermonthica* and *Orobanche crenata*. Our results demonstrate that the germination stimulants of *S. hermonthica* present in the root exudates of maize, cowpea and sorghum are derived from the carotenoid biosynthetic pathway. Based on these results, we have postulated a biogenetic scheme for the strigolactone germination stimulants.

## **The Influence of *Arceuthobium pusillum* Infection on the Hydraulic Architecture of White Spruce Stems**

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*Arceuthobium pusillum* (eastern dwarf mistletoe) is an aerial hemiparasitic angiosperm whose primary hosts are white spruce (*Picea glauca*), red spruce (*Picea rubens*) and black spruce (*Picea mariana*). Although the biology, ecology, and population genetics of *Arceuthobium* species have been examined in some depth, comparatively less is known about the impacts of *Arceuthobium* infection on the physiological performance of their hosts. *Arceuthobium* infection is characterized by the establishment of an endophytic system that taps directly into host xylem and phloem, through which the parasite withdraws all of the water, mineral nutrients and much of the fixed carbon that it needs to support its growth. Along the coast of Maine, *Arceuthobium pusillum* infection causes severe mortality in white spruce stands. We investigated the impact of *Arceuthobium pusillum* infection on white spruce stem hydraulic conductivity. Infection reduced white spruce stems' capacity to deliver water (specific conductivity) by more than 25%. This perturbation may explain the dramatic reduction (>40%) in needle size distal to infection. Needle size reductions may offset reductions in hydraulic conductivity and water use by *Arceuthobium* to restore the balance between host leaf area and the capacity to transport water through stems. As a result, infection had no significant impact on a stem's capacity to deliver water when expressed on the basis of leaf area distal to infection (leaf specific conductivity). Furthermore, we found that infection did not have an impact on the occurrence of cavitation in white spruce stems.

## **Significance of Parasitic Weeds for Food Legumes in Syria**

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Parasitic weeds, especially *Orobanche* and to a lesser extent *Cuscuta*, have been major constraints for many crops in the Middle East, particularly legumes such as lentil, faba bean, and recently winter-sown chickpea. Losses due to parasitic weeds are usually poorly documented mainly due to misleading survey results based on the observations of the parasite in the field rather than on the seed bank in the soil. Extensive research on crop and weed management in Syria, including crop rotations, fertilizers and trap crops as cultural control practices have had no impact on *Orobanche* alleviation. Delayed planting date is the only cultural practice available to the farmer to reduce *Orobanche* infestation, but this practice also reduces crop yield potential. Breeding for *Orobanche* resistance has had only limited success, except with developed Egyptian faba bean varieties. Synthetic stimulants did not perform *in vivo* as they did *in vitro* due to calcareous soil, and did not either reduce the seed bank or the level of parasite infestation. Solar sterilization using second-hand plastic sheets is having an increasing role in reducing infestation levels of weeds, including *Orobanche*, and other pests, under greenhouse vegetable production in extensive farming of coastal regions of Syria. Chemical control of parasitic weeds by herbicide application in some crops appears promising; however, more research is needed to minimize crop injury and to widen the herbicide selectivity for up-scaling recommendations to other crops. Despite available recommendations for chemical control of parasitic weeds in faba bean, and recently in lentils, adoption by farmers is low due to lack of effective extension. While much has been learned about the evolution, physiology, genetics, and ecology of parasitic plants in the past few decades, economic crop losses remain unacceptably high in most countries of the Mediterranean region. Concerted efforts are needed to apply at farm level what is already known about parasitic weeds in food legumes.

## **ROS production and Semagenesis in Pathogenesis**

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The potential of reactive oxygen species (ROS) such as superoxide ( $O_2^{\cdot-}$ ) and hydrogen peroxide ( $H_2O_2$ ) to induce oxidative stress (damage) in biological macromolecules is well established. While ROS production is well-known as a byproduct of oxidative metabolism and co-opted by eukaryotes as a common component of the response to wounding and/or infection, these potent oxidants have recently been implicated as signaling molecules in a number of critical developmental events. In the parasitic plant *Striga asiatica*, ROS regulates host detection and organogenesis of the haustorium, a specialized organ for host attachment. *S. asiatica* seedlings produce  $H_2O_2$  which serves as the co-substrate with host cell wall phenols and apoplastic peroxidases to release haustorial inducing p-benzoquinones in a process we have named semagenesis. Employing a variety of  $H_2O_2$ -responsive stains in conjunction with confocal, fluorescence, and transmission electron microscopy, we have localized the site of  $H_2O_2$  production and characterize its regulation. These results support a model in which ROS generated by the parasite is a critical component of the signaling process and required for haustorial organogenesis. We therefore suggest that ROS may function in a semagenic role in the oxidation of cellular components in other organisms, perhaps in conjunction with other small molecules or enzymes, generating secondary signaling products.

## **Gene Regulation during Haustorial Development and Shoot Initiation in *Striga asiatica***

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In the parasitic angiosperms the vascular connection with the host occurs via formation of a specialized organ known as the haustorium. In *Striga asiatica*, a parasite of grasses, haustorial organogenesis is regulated by small molecules released from the host. Methods have now been developed for subtraction library screening of these obligate parasites. We now report the successful identification of two lignin biosynthetic genes -- chorismate mutase (StCM1) and cinnamyl alcohol dehydrogenase (StCAD1). The first StCM1 is the initial step in phenylalanine, tyrosine, and ultimately lignin biosynthesis, while the later StCAD1 is the last step in the formation of lignin monomers. The expression levels of both genes increases early in haustorial induction and remains elevated throughout organogenesis. The promoters of these two genes resulted in GUS expression specifically in the vascular tissue of *Arabidopsis thaliana*. Over-expression of StCM1 resulted in significant morphological changes, with the most remarkable change being a 50% increase in the number of stem xylem bundles. We discuss the possible reasons for this significant alteration of the *Arabidopsis thaliana* development program in the context of haustorial development. Using the same approach, we induced shoot initiation of *Striga Asiatica* by trans-zeatin, and constructed differential cDNA library to characterize the transcript profile that regulates shoot initiation.

## **Development of a Biological Herbicide for Control of *Cuscuta* spp.**

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On May 18, 2005 the U.S. Environmental Protection Agency published a regulation that established an exemption from the requirement of a tolerance for residues of the microbial pesticide *Alternaria destruens* Strain 059, marketed commercially as Smolder\_, on all agricultural commodities when applied and used in accordance with label directions for the control of dodder. This represented the culmination of 20 years of research but the beginning of efforts at commercial development. Large scale demonstrations were conducted in 2006 in several states in the U.S. with both a granular and a wettable powder formulation. Success was mixed. In Wisconsin cranberry fields, dodder control ranged from 52 to 100 per cent. In Massachusetts cranberry fields, dodder control ranged from 20 to 60 per cent. In Iowa carrot fields, dodder control ranged from 0 to 100 per cent. Differences in climatic conditions and, possibly, the genetic makeup of the dodder populations tested could account for this variability.

## **Induced Host Resistance as a Control Method for Parasitic Weeds**

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Parasitic weeds restrict crop production in many countries. Plant induced resistance is against not only pathogens and herbivores, but also parasitic weeds. Induced resistance against parasitic weeds has been demonstrated in the associations of sunflower-*Orobancha cumana*, tobacco/hemp-*O. ramosa* and pea-*O. crenata* under greenhouse conditions and/or in the field. Plant activators include biotic and abiotic agents. Host plants underlay systemic acquired resistance (SAR) or induced systemic resistance (ISR) pathway against parasitic weeds. Induced resistance should be integrated in the parasitic weed management systems. This paper reviews the history and development, pathway and mechanism, as well as prospect and outlook of plant induced resistance on the control of parasitic weeds.

## **Agrobacterium-Mediated Transformation of *Striga hermonthica***

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*Striga* spp. (witchweeds) are major agricultural pests which infest important crops in sub-Saharan Africa and parts of Asia. To develop control methods against *Striga* infestation, understanding the molecular basis underlying its parasitism is of particular importance. However, molecular knowledge about *Striga* infection is still limited. Reverse genetic approaches are powerful tools to assess unknown gene function and identify functional genes. Establishment of a transformation system is a critical step to conduct reverse genetic experiments. In the present study, we aim to establish transformation methods of *Striga hermonthica* using *Agrobacterium*. *Striga* is an obligate root parasite which is unable to live without its host in natural conditions. Therefore we initially developed axenic culture conditions of *S. hermonthica*. Surface sterilized *S. hermonthica* seeds were germinated and grown under axenic conditions. *Striga* plants were able to develop plant architecture with shoots and root-like structures in the presence of sufficient nutrients and carbon source in the media. Callus formation and emergence of shoot and root-like structures were controlled by a balance of auxin and cytokinin. We tested transformation of aseptically grown *S. hermonthica* via *Agrobacterium tumefaciens* or *A. rhizogenes* using green fluorescence protein (GFP) as a marker. Our preliminary results showed that *A. rhizogenes*-mediated transformation successfully generated GFP expressing hairy roots of *S. hermonthica*.

## **A Study of Biodiversity of African *Radopholus Similis* In Uganda.**

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The main problem that nematologists are facing during screening for nematode resistance is the variability in the reproduction of different *R. similis* populations. Assessment of this variability will be highly useful to banana breeding programs for incorporating resistance to nematodes. The reproductive fitness of four *Radopholus similis* populations (Ikulwe, Sendusu, Mukono and Mbarara) were studied on carrot disc (in vitro) and on plantain cultivar (in vivo) *Obino / Ewai*, as a function of time, inoculum densities and its pathogenicity on plant. In the first experiment, the reproductive fitness of the isolates was followed during two, four, six and ten weeks after inoculation. There were significantly ( $p < 0.05$ ) differences in the reproductive fitness of *R. similis* populations from Uganda as an effect of time. Increase in time increases the final nematode population. The experiment to test the effect of different inoculum density with respect to the reproduction of these populations on the carrot discs shows no significant difference in their rate of reproduction at 6 weeks after inoculation. Regarding reproduction and pathogenicity of *R. similis* on plants, no definite conclusion can be drawn from the result obtained.

**AO – 548, a Sunflower Inbred Line, Carrying Two Genes for Resistance Against a New Highly Virulent Romanian Population of *Orobanche cumana*.**

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A new highly population of the parasite *Orobanche cumana* has attacked sunflower crop in Romania, in 2005 year. In the sunflower germplasm collection of Fundulea institute has been identified a restorer line, AO – 548, full resistant to this new broomrape population. Since this line could be used directly as a parent to produce commercial F1 hybrid seed, as well as a source of resistance to broomrape in sunflower breeding program, it was decided to study the inheritance of resistance in this line, to the new population of *Orobanche cumana*. Using the cytoplasmic male sterile inbred line AD – 66, known to be very susceptible to this population of the parasite, as a female parent, progenies of a cross with AO – 548 (including F1, F2, and BC1 to both parents), as well as the parental lines, were analyzed for their reaction to this broomrape population. The goodness of fit of the observed vs expected segregation ratio (15:1 and 3:1) indicated that the inheritance of resistance to broomrape in the line AO – 548 is conferred by two independent dominant genes.

**Colonization of *F. oxysporum* f.sp. *strigae* (Foxy 2) on roots of sorghum plants and its implication for *Striga* control using a seed treatment delivery system: an anatomical study**  
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The application of biocontrol agents by a seed-coating procedure is an attractive option for establishing the biocontrol agent in the rhizosphere, the infection zone of the root parasitic weed *Striga* and offers a simple delivery system. *Fusarium oxysporum* f.s. *strigae* (Foxy 2) showed promising potential in controlling *Striga* when applied as a film-coat on sorghum seeds. The ability of Foxy 2 to colonize the root system of sorghum seedlings that emerged from film-coated seeds, as a prerequisite, was proved by plating pieces of roots on PDA medium. In addition, anatomical investigations were performed to follow the colonization of sorghum roots by Foxy 2. The spreading of the mycelia were observed by using light microscopy 1, 2, 3 and 4 weeks after germination. Transmission electron microscopy was used to get more information about the interaction between Foxy 2 and the roots of sorghum. Foxy 2 acts as a pathogen to *Striga* but is non-pathogenic to other plants. Nevertheless, Foxy 2 was growing on the root surface and penetrating the roots of sorghum. Within 2 to 3 weeks it was slowly colonizing the whole cortical parenchyma of the root, but hyphae did not enter the vascular cylinder and kill the plants even after 4 weeks. On the contrary, Foxy 2 even seems to promote the growth of sorghum plants. This indicates that Foxy 2 met the criteria of being an effective mycoherbicide for controlling *S. hermonthica* using seed treatment technology.

## **Synergy Between *Striga*- Mycoherbicides “*Fusarium oxysporum* f.sp. *strigae*” and Resistant Cultivars Under Field Conditions: Step Towards Integrated *Striga* Control in Africa**

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*Striga* spp. are important constraints in cereal and legume production in semi-arid tropical Africa. An integrated approach, in which biocontrol represents an important component, appears to be a promising strategy for reducing *Striga* infestation. *Fusarium oxysporum* f.sp. *strigae* (isolates Foxy 2 & PSM197) are potential, highly host specific mycoherbicides against *S. hermonthica*. For facilitating practical field application, our recent research focuses on the development of appropriate mycoherbicidal formulations and delivery systems. Hence, Pesta formulation made by encapsulating fungal inoculum in a matrix composed of durum wheat-flour, kaolin, and sucrose, was developed. Seed treatment technology for coating sorghum and maize seeds as an attractive option for further minimizing the inoculum amount and facilitating delivery of *Striga*-mycoherbicides was also provided. Integration of Pesta formulation and treated seeds containing *Striga*-mycoherbicides (Foxy 2 & PSM197) with *Striga* resistant and susceptible maize and sorghum cultivars under field conditions of West Africa was investigated. The combination Pesta granules or treated seeds and resistant maize and sorghum cultivars enhanced clearly both mycoherbicides efficacy, and showed the strongest suppressive effect on *Striga* compared to susceptible cultivars. The difference between the resistant and the susceptible cultivars was stronger for maize than for sorghum. On average (i.e. the average effect of both isolates), they reduced the number of emerged *Striga* plants per plot by 96% or 89% when the two isolates formulated as Pesta granules or delivered as coated seeds, respectively, and combined with the resistant maize cultivars compared with the control of the susceptible cultivars. On the other hand, the respective reductions when the two isolates integrated with the susceptible maize cultivars were 85 %, and 21%. Improvement in maize and sorghum performances (height, grain yield, stalks dry weight, etc.) was recorded. Further, both mycoherbicides maintained excellent viability (shelf-life) on Pesta products and treated seeds after one year of storage which would be sufficient for their use under practical conditions of storage, handling and delivery. The suitability of Pesta and seed treatment technology for formulating and delivering *Striga*-mycoherbicides and their compatibility and synergy with *Striga* resistant cultivars, are highly relevant to the realization of an integrated *Striga* control approach adoptable and applicable by subsistence farmers in Africa.

**Key words:** *Weed biological control, Mycoherbicide, Encapsulation, Seed coating, Integrated control, Fusarium oxysporum, Striga hermonthica, Striga-resistant cultivars, Sorghum bicolor, Zea mays*

## **Molecular Analysis of *Lotus japonicus* Response against *Orobanche aegyptiaca* and *Striga hermonthica* Parasitism**

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Root parasitic plants directly invade and rob water and nutrients from host plants and thus cause major constraints to crop production in many parts of the world. *Lotus japonicus*, a model leguminous plant that is compatible to the holoparasite *Orobanche aegyptiaca*, but incompatible to the hemiparasite *Striga hermonthica*. In order to understand host responses at early stages of parasitization at a molecular level, we established cDNA libraries enriched by suppression subtractive hybridization (SSH) for transcripts regulated in *L. japonicus* root segments adjacent to *O. aegyptiaca* tubercles (LjOa) and *L. japonicus* root segments adjacent to the attachment points of *S. hermonthica* seedlings (LjSh).

Following sequencing and expression analyses, we found that 59 and 46 genes were up-regulated in the LjOa and LjSh libraries, respectively. Both of the libraries were enriched for transcripts predicted to function in stress responses including PR proteins and enzymes involved in jasmonic acid biosynthesis. Transcripts associated with nodulation were significantly more abundant in the LjOa library compared with the LjSh library, while those associated with phytoalexin biosynthesis were found exclusively in the LjSh library. These results suggest that the compatible parasitic plant deceives the host plant into lowering resistance at early stages of parasitization.