EUROPEAN SCIENCE FOUNDATION
SETTING SCIENCE AGENDAS FOR EUROPE

Exploratory Workshop Scheme

Standing Committee for Life, Earth and Environmental Sciences (LESC)

ESF Exploratory Workshop on

Plant-Microbe-Insect interactions: from molecular mechanisms to ecological implications

Wageningen (The Netherlands), 18-20 August 2011

Convened by:
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SCIENTIFIC REPORT

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1. Executive summary

Practical organization

The meeting was held in Hotel “De Wageningse Berg” in Wageningen, NL, starting the morning of Thursday 18 August and ending the afternoon of 20 August, 2011. There was a total of 23 participants from 12 different countries, plus a rapporteur of ESF. Since the location serves both as a hotel and as a conference centre, it conveniently provided all the facilities needed at a single location. The centre is located at the edge of a small forest at one of the highest points of Wageningen and has a large terrace with a magnificent view overlooking the river Rhine, which contributed to the open and pleasant atmosphere during the workshop. Participants widely appreciated the diversity of disciplines and researchers at different stages of their carrier that were present at the workshop and all felt very stimulated by the presentations, discussions and contacts that they made.

Scientific objectives

Plants are intimately associated with both microbes and insects. This gives rise to three-way interactions that have far-reaching consequences for the functioning and evolution of the organisms involved. After the initial disparate development of the fields of plant pathology (focusing on plant-microbe interactions) and entomology (focusing on plant-insect interactions), the past decades have seen the rise of an integrated field of plant-microbe-insect interactions, which has recently been revolutionized by advanced molecular studies. Fascinating insights have emerged since these new developments have allowed researchers to address questions like: how do plant microbial symbionts/antagonists affect interactions between plants and herbivorous insects and vice versa? To what extent are the patterns of activation of defense signalling pathways shared between different guilds of microorganisms and insects? Are there predictable patterns in synergistic and antagonistic interactions between these players? How is the behaviour and performance of herbivorous insects altered by their endosymbionts/ pathogens and what are the consequences of these alterations? The current challenge is to understand the ecological, evolutionary, and agronomic relevance of these interactions and their role in the functioning of plants in their multitrophic environment.

The aim of the workshop was to bring together and stimulate cross-talk between molecular and ecological researchers from this emerging field to give impetus to future research on mechanisms, patterns, and ecological, evolutionary, and agronomic implications of such three-way interactions. The workshop thus included experts on plant-associated microbes (mycorrhizae, rhizobia, plant growth promoting rhizobacteria, endophytes, oomycete/ viral/ fungal/ bacterial pathogens) and insect-associated microbes (endosymbionts, entomopathogens), and their effects on herbivorous, pollinating and vectoring insects, and vice versa. Specific aims were to facilitate participants to explore new research areas and future collaborations, explore potential topics for joint papers, develop research plans to be submitted to the EU or other funding agencies, and initiate steps to set up a European research network on plant-microbe-insect interactions.

Workshop agenda

The workshop consisted of three scientific sessions and a fourth session during which follow-up activities were discussed. The three scientific sessions were organized around the themes (1) patterns of interactions from mutualism to antagonism across systems (2) molecular mechanisms of interactions, and (3) ecological, evolutionary and community consequences of interactions. The scientific sessions consisted of a total of twenty short (25 min) talks. Speakers had been urged in advance to devote some time in their presentation to go a step beyond their specific research results and express their views on knowledge gaps and (cross-disciplinary) areas that need to be developed, and to fuel discussion.

Two boards were put up in the meeting room on which all participants could stick notes after each of the talks. One board collected ideas on “knowledge gaps”, the other on “applied aspects and funding opportunities”. The evenings were devoted to plenary discussions in the meeting room starting from themes emerging from the collected notes. Thereafter, discussions could be informally continued on the terrace with a drink.

The sunny afternoon of the second day was devoted to a walking excursion through nature reserve “The Blauwe Kamer”. This gave participants the opportunity to extend their informal contacts and to discuss ideas and opportunities for collaborations while enjoying the scenery of the river embankment area and its flora and wildlife.

Conclusions and follow-up activities

The workshop concluded that research on Plant-Microbe-insect interactions is important for understanding the functioning, ecology and evolution of plants and their associated community. Microbes often appear as “hidden players” in plant-insect interactions and vice versa, but often even qualitatively alter the outcome of such interactions. Applied aspects of these interactions include conservation (e.g. preserving not only species but also key interactions), invasion biology (e.g. preventing spread of indigenous diseases by introduced vectors) and food security (e.g. manipulating insect endosymbionts to reduce the competence of disease vectoring insects, the use of benefical plant microbes to prime plants defenses against insect pests or enhance the attraction of their natural enemies).

The workshop identified a number of knowledge gaps. (1) While for some groups of organisms (e.g. effects of mycorrhizae or rhizobia on plant-insect interactions) we begin to understand patterns of interactions and their mechanisms, for others (notably endophytes) this is in its infancy. (2) Molecular studies begin to unravel how plants tailor their responses to microbes and insects. Phytohormones that are key players in induced defenses have been identified and reveal shared patterns of defense signaling pathways triggered by particular guilds of insects and pathogens. But the role of other hormones in interactions with specific groups of microbes and insects, how these interactions are modulated by the abiotic environment, and the specificity of resonses arising from downstream activated defenses are poorly understood. (3) The outcome of plant-microbe-insect interactions can vary from beneficial to detrimental even for players within a single system. We need to understand factors underlying this variability. (4) Perhaps the most notable gap in our knowledge is understanding the significance of three-way interactions in a community context, i.e. what are the consequences of interactions for the composition and dynamics of the associated community (rather than on individuals) These areas should be part of the future research agenda.

As follow-up activities the participants agreed to (1) submit an application to the EU for a COST action in the domain of Food and Agriculture to coordinate and stimulate research in this area (submitted Sept 30, 2011), (2) Apply for a follow-up conference at the Universidad Internacional de Andalucia, (3) Submit a proposal for a journal special issue on Plant-Microbe-Insect interactions, and (4) Launch a website as a platform for researcher in this field (https://sites.google.com/site/plantmicrobeinsect/).

2. Scientific content of the event

Below is a brief summary of each of the presentations, including the discussions and views on the directions that the field should take, that emerged from them.

The programme started with a general introduction. **Arjen Biere** gave a brief historical overview of the emergence of the field of Plant-Microbe-Insect interactions from the fields of plant pathology and entomology. He ascribed the recent boost of the field to (1) the revolutionizing insight in cross-species induced resistance from recent molecular studies, (2) the broadening of the field, expanding from pests and pathogens to the whole diversity of beneficial and detrimental microbes and insects, and (3) the broadening of the field from species to community studies. **Thure Hauser** explained the aims of the workshop and stressed the exploratory nature of it, necessitating that participants to reserve time to express their views on the field during the presentations. He also urged participants to use the boards to attach notes with (1) views on areas that need to be explored and (2) views on applied aspects and funding opportunities to fuel the evening discussions. **Wolfgang Weisser,** present as the representative of ESF, explained the role of ESF in the European research arena and outlined opportunities for follow-up activities after the explanatory workshop. He explained that ESF is currently streamlining its activities in line with the needs of its Member Organizations, reason why RNP and EUROCORES theme calls have temporarily been postponed.

Session 1 was devoted to an inventory of patterns of beneficial and harmful effects that can be observed in Plant-Microbe-Insect interactions, when looking across the whole breath of systems. Below, the contributions are grouped by the type of microbe involved in the interaction. First, contributions involving effects of *plant-associated microbes*: mycorrhizae, plant growth promoting rhizobacteria (PGPR), rhizobia, whole soil biota, plant endophytes and plant viruses, followed by contributions involving effects of *insect-associated microbes*, specifically insect endosymbionts.

Alison Bennett summarized studies of effects of **arbuscular mycorrhizal (AM) fungi** on plant trophic interactions with pollinators, herbivorous insects and natural enemies of herbivores. She showed that the patterns stongly depend on insect feeding strategy and degree of host specialization. AM fungi generally reduce performance of generalist *leaf chewing* insects (caterpilars) but increase the performance of generalist *phloem* feeding insects (aphids), while having less effects on specialist insects. A variety of mechanisms is involved, including changes in plant size, nutritional quality, induced defenses, tolerance, and plant volatiles. She stressed that all studies thus far have involved only a handful of AM species whose effects have usually been studies in isolation, while plants never associate with a single AM species. The field needs studies with a broader array of AM species to confirm the generality of results, and with whole AM communities to elucidate effects under more realistic conditions, and to assess effects of changes in soil microbial communities associated with e.g. altered land use on plant interactions with the aboveground insect community.

Ana Pineda addressed the role of beneficial rhizobacteria in plant-insect interactions. Non-pathogenic microbes like mycorrhizae and **plant growth promoting rhizobacteria (PGPR)** enhance plant growth but can also induce systemic resistance to pathogens and insects through priming. Patterns of effects on insects are remarkably similar for mycorrhizae and PGPR. She showed that PGPR enhances performance of a generalist (but not a specialist) *phloem* feeding insect and reduces the attractiveness of plant odours to a parasitoid natural enemy of the generalist phloem feeder. She stressed the importance of studying the balance between positive (growth promoting) and negative (in this case enhanced pest) effects of PGPR on plants. She also stressed the need for integrated studies of effects of PGPR on a broader array of plant hormones to understand modulation of PGPR effects by the abiotic environment. For instance, PGPR prime plants for defense responses mediated by jasmonic acid, but suppress absciscic acid, involved both in induced systemic resistance and drought tolerance.

Anne Kempel addressed the effects of both mycorrhizae and **rhizobia** on plant-herbivore interactions. She used clover lines that were or were not impaired in their ability to associate with nodulating rhizobia to show positive effects of rhizobia on both clover growth and on generalist leaf chewing (but not phloem feeding) insect performance, contrasting with effects commonly observed for AM fungi and PGPR. She stressed the importance of plant genetic background for the outcome of three-way interactions: positive effects of rhizobia on the generalist chewing insect were only observed in strains that lacked the ability to defend themselves by nitrogen-containing defense compounds (cyanogenic glycosides). She also stressed the importance of the timing of insect and herbivore attack on the outcome of three-way interactions. Mycorrhizae increased plant production and enhanced performance of generalist insects, but only if plants had not been induced by the herbivore four weeks prior to attack; moreover these effects were species-dependent. She advocated a multi-species approach to assess the generality of observed patterns.

Eduardo de la Peña addressed effects of soil biota on plant-pollinator interactions and on evolutionary adaptation in aboveground herbivores. Restoration of heathlands from former agricultural fields affects interactions of heather with both ericoid mycorrhizae and pollinators. Restored heathlands have lower mycorrhizal colonization than reference heathlands, causing them to flower later and have fewer pollinator visits, lowering their reproduction. This signifies the importance of soil microbes for plant-pollinator interactions. He also showed that soil biota can play an important role in the evolution of aboveground herbivores. During a multi-generation selection experiment, spider mites became **locally adapted** to the biotic soil conditions of their host plants (i.e. presence or absence of mycorrhizae and plant-feeding nematodes). That is, they performed better on hosts grown under the soil biotic conditions that they experienced during selection. This illustrates that arthropods are able to adapt to the specific plant phenotype that is induced by soil biota. Similarly, field studies suggest that aphids can adapt to the local mycorrhizae of their host plants. The talk fueled much of the evening discussion on the significance of evolutionary adaptation in plant-microbe-insect interactions and who are the drivers in this process.

Jochen Krauss addressed whether effects of **grass endophytic fungi** on herbivores and on higher trophic levels are predictable. Endophytes are endosymbiont bacteria or fungi that live within plants without causing apparent disease symptoms.They are hyperdiverse but their function is largely unknown. Only the small group of host-specialized (“class 1”) endo-phytes that colonize grass species has been relatively well characterized. Ergot alkaloids and other products of this endophyte class in grasses cause significant economic losses due to effects on livestock, but also increase grass protection from insects. These endophytes can increase grass nutrition, drought tolerance and herbivore resistance, but the effects are strongly context dependent. He showed that both aphids and their predators and parasitoids perform worse on endophyte-infected grasses, when studied in a greenhouse. Strikingly however, effects were much less pronounced to absent in field studies, and marginal compared to effects of fertilizer and cultivar. He argued that this calls for shifting studies towards effects of these microbes in a more realistic and complex food web setting.

Stefan Vidal addressed how **non-specialized endophytic fungi** mediate plant-herbivore interactions. The majority of non-host specific endophytes (“class 2-4”) have unknown distributions and effects. He illustrated the enormous diversity of seed- and soilborn endophytic fungi. One grass species may harbour endophytes from over a hundred different fungal taxa, their abundances may show dynamic patterns even within a season, and a single species may act as a pathogen in some hosts, but as antagonist of pathogens in others. He illustrated the potential of these endophytes to reduce pests using studies of one of the non-specialized endophyte genera, *Acremonium*. These endophytes alter the leaf voltile profile of tomato, broad bean and brussels sprouts, enhancing their attractiveness to adult lepidopteran herbivores, but they reduce the plant's suitability for the offspring of these herbivores because they increase levels of toxic secondary metabolites and decreases levels of phytosterols that are precursors of moulting hormones in insects, preventing their proper moulting. He stressed the need to assess the diversity and effects (from mutualism to antagonism) of this large group of ubiquitous, less specialized, endophytes with their enormous potential to moduate plant-insect interactions.

Benedicte Albrectsen addressed how we can study the beneficial potential of non-specialized **fungal endophytes of trees** and use them as guards and source of novel biomolecules. She described how molecular techniques are now used to survey the ecological function of endophytes in aspen clones. Over a thousand isolates were distinguished by traditional methods, representing more than a hundred morphologically distinct groups. For a dozen taxa that were distinguished based on DNA-sequences from these groups, primer pairs were designed that allowed endophytes to be identified in field samples, with a very acceptable detection limit. She showed that the presence of some of these endophytic fungi in aspen clones was significantly related to field damage by herbivores. There was also an association between endophytes and condensed tannins, but is is yet unclear whether that association is causal or not. She stressed that this approach now allows qualitative and quantitative detection of endophyte communities, and to elucidate their ecological function. She stressed that this knowledge is needed to understand why endophytes remain asymptomatic, and what determines whether their association becomes mutualistic or antagonistic for the plant.

Scott Johnson addressed how plant microbes influence host plant location by insect herbivores. Plant microbes can alter the attractiveness of their hosts, and thereby alter host plant location by insect herbivores, even to the extent that non-hosts can be turned into hosts. **Viruses** can alter the volatile profiles of their host plant, affecting attraction of their vectors. He argued that in order to understand effects of virus-infection on host plant quality we need to take the virus transmission strategy into account. In persistent viruses, that show sustained feeding from the phloem, acquisition by their vector takes long (hours to days). He expected that this should select for alterations that improve the plant quality for the vector, as the virus needs to attract the vector and keep it feeding. By contrast, in non-persistent viruses, that just probe plants with their mouth parts, acquisition by their vectors takes only seconds. This should select for alterations that decrease host plant quality for the vector, as the virus needs to attract the vector but make sure it moves on quickly to maximize transmission. He showed that experimetal data are grossly in line with these predictions, offering an explanation for the patterns that emerge from recent studies on how viruses affect host plant quality for insect herbivores.

Enric Frago gave an overview of effects of **insect endosymbionts** on plant-insect interactions in a mutitrophic context. Insect symbionts include bacteria, fungi and protozoans. Primary (obligate) endosymbionts are essential for survival of the host whereas secondary (facultative) endosymbionts enable a diversity of extra functions in their hosts. Their services to insects include enhanced nutrition, immune responses, avoidance of plant defenses, and protection from natural enemies. He showed that the strenght and direction of their effects depend on genotype and species of the endosymbiont. He stressed that insect symbionts are extremely important as they facilitate phenotypic innovations of their hosts, leading to increased diversification and host use. For instance, Pea aphids have seven facultative symbionts, that affect the range of plant species that their hosts can use, and that protect these hosts from pathogens, parasitoids and heat shocks. Experiments in which aphids are experimentally infected or cured show that the endosymbionts increase aphid fitness on their preferred host plant but reduce fitness on alternative host plants, reinforcing host plant specialization. Importantly, he showed that these effects cascade up across trophic levels. For instance, symbiotic fungi of bark beetles increase their host's growth rate, and facultative endosymbionts of whiteflies enhance the transmission of their vectored viruses. He stressed the occurrence of such community-wide effects resulting from endosymbionts, often "hidden" players in the field.

David Giron addressed how **endosymbionts of leaf miners** trigger host plant physiology to the benefit of their host. Leaf miners selectively feed within leaves on more nutritious and less defended tissues. Mined areas contain high levels of cytokinins (green islands), retarding senescence and associated yellowing of the leaf. By manipulating the presence of leaf miner endosymbionts using antibiotics, he could show that it is not the leaf miner itself but its *Wolbachia* endosymbionts that play a crucial role in this sustained production of cytokinins. They create the green islands in otherwise senescing tissue by inducing changes in the expression levels of cytokinin receptors in the plant. The sugar content in green islands on senescing leaves is enhanced at the expense of the sugar content of mined areas in green leaves, providing a more constant carbon source to the larvae. Wolbachia also mitigates increases in phenolic compounds (flavonoids) in ageing leaves. This all strongly enhances overall larval success of the leaf miner. Phylogenetic studies confirm the association between Wolbachia symbiosis and the ability to form green islands. He stressed that these results show that insect endosymbionts can manipulate plant physiology and reconfigure plant metabolism to the benefit of their hosts, but he urged the need for studies of effects of endosymbionts at higher trophic levels.

The **evening discussions** that emanated from this session centred around a few themes: (1) Clear patterns in the outcome of plant-microbe-herbivore interactions can be observed from studies of effects of microbes that are considered as plant mutualists (mycorrhizae, plant growth promoting rhizobacteria) on different guilds of herbivores, but studies on another potentially very important group of microbes (endophytes) is still in its infancy; this should be one of our priorities. (2) Studies are now beginning to reveal evidence for evolution and local adaptation in plant-microbe-insect interactions, but we need combined efforts to discover who are the drivers in these processes. (3) Studies of plant-microbe-insect interactions are still too much focused on specific interactions involving few species; the field should evolve in the direction of a community context, assessing both community effects and community consequences of plant-microbe-interactions (4) The outcome of plant-microbe-insect interactions is plastic, ranging from beneficial to harmful for players involved even in a single system. There is an urgent need of studies on factors, such as environmental (abiotic) conditions, that are important determinants of the variability in outcomes, and on who benefits from the interaction.

Session 2 was devoted to insights from molecular and biochemical studies into the mechanisms underlying plant-microbe-insect interactions. The presentations focused on (1) molecular mechanisms involved in induced resistance and priming of defense responses of plants, (2) mechanisms by which plant viruses hijack the plant's metabolism to enhance their transmission by insect vectors or by which they affect interactions with insect pests, and (3) the contribution of combined genomic and metabolomic approaches to study plant-microbe-insect interactions.

Corné Pieterse addressed how beneficial and parasitic microbes and insects modulate plant immunity in the model plant species *Arabidopsis thaliana*. Plant defenses against pathogens and insects are regulated by a limited number of cross-communicating signaling pathways in which salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) play key roles. He showed that there is now good evidence for considerable overlap between transcriptional changes that are induced by pathogens and by insects, with very different modes of attack. For instance, of all the consistent changes induced by a biotrophic leaf fungus, a leaf chewing, phloem feeding, and cell-content feeding insect, more than half is shared with those induced by a growth promoting rhizobacterium. However, he stressed that even though these attackers all stimulate JA biosynthesis, the majority of (downstream) changes in JA-responsive gene expression are attacker specific. Thus, even though clear general patterns emerge at the level of signaling, there is still complex variation at the level of downstream attacker-specific defense responses, that is shaped by other regulatory mechanisms. This downstream variation makes it difficult to predict patterns in plant-mediated interactions between guilds of microbes and guilds of insects simply based on their signaling profiles. He stressed the need to study how plants prioritize their defenses in multiple systems, i.e. expanding work from Arabidopsis to other model systems.

Maria Pozo addressed molecular aspects of the impact of arbuscular mycorrhizas on aboveground plant-insect interactions. AM fungi only transiently affect levels of salycilic acid (SA) and jasmonic acid (JA) in plants. Initially, SA is upregulated, followed by downregulation as the symbiosis develops, suggesting that AM fungi suppress SA mediated responses. This is often followed by a transient upregulation of JA. However, most evidence now points to a model in which AM fungi do not not directly activate JA defenses, but *prime* the plant for JA-dependent defenses, i.e., amplify JA responses triggered by herbivores, resulting in reduced performance of generalist leaf chewing insects that are signaled by JA. Her recent studies have identified a small peptidic hormone as a potential candidate for mycorrhizal priming of JA defenses in tomato. She showed that AM fungi upregulate this hormone in tomato, that it results in priming of JA dependent responses, and that caterpillars of tobacco hornworm perform worse on AM plants and tomato lines with constitutive expression of this hormone compared to lines in which its expression has been silenced. She stressed that further studies are required to reveal whether this hormone is indeed the major player in AM-mediated priming in tomato, and to reveal mechanisms of AM priming in other plants.

Eduardo Bejarano addressed how geminiviruses inhibit jasmonate signaling, the hormones that mediate plant defense against generalist leaf chewing insects. Plant viruses generally create a proper environment for their replication, spread and transmission by suppressing plant defenses and by hijacking cellular machinery for viral functions and transmission. He unraveled in molecular detail how tomato yellow leaf curl virus (TYLCV), a geminivirus that is transmitted by whiteflies, inhibits JA signaling in *Arabidopsis thaliana*. The JA signaling cascade is normally initiated when wounding by herbivores increases levels of jasmonates, leading to the degradation of JAZ proteins that suppress MYC2, the key transcription factor initiating the JA signaling cascade. The degradation of JAZ proteins is mediated by their binding to a protein complex that catalizes their ubiquitination (the process by which proteins are destined for degradation by proteasomes). Viral C2 proteins sabotage degradation of JAZ proteins by interacting with host proteins involved in binding of these JAZ proteins to the complex that catalizes their ubiquitination. Geminiviruses thus suppress jasmonate responses, which in turn accelerates the insect vector's cycle because nymphal development is no longer suppressed by JA responses, enhancing viral spread.

Stéphane Blanc showed how plant viruses can sense the presence of their insect vectors on an infected host and immediately transform into a transmissible morph. Cauliflower mosaic virus (CaMV) infects turnip and is transmitted by aphids. The general view has been that viruses are randomly distributed across plant cells and that their acquisition by aphid vectors is a random process. However, viruses are concentrated in transmission bodies. He unraveled how aphids can "find" these transmission bodies so efficiently. Transmission bodies go through different forms during aphid feeding. An important finding was that upon aphid feeding, tubulin massively accumulates in transmission bodies. Tubulins are proteins that make up microtubules, rope-like polymers that are e.g. highways for intracellular transport. Tubulin entry marks the activated state of the transmission body and this activation is required for successful transmission. The entry of tubulin in transmission bodies is triggered by aphid stylet penetration, and is extremely fast. Only transmission bodies close to the salivary sheaths that are left behind by aphid feeding are activated. The activated form then transforms into an exploded form (tubulin network formation) that is necessary for functionality of the transmission bodies. The virus thus hijacks the perception system of the plant to respond to its vector presence and location to enhance its transmission.

Kristina Gruden addressed the molecular basis of potato interactions with potato virus Y (PVY) and the Colorado potato beetle (CPB). She showed that plant responses to PVY alone strongly depend on potato variety. A moderately resistant variety upregulates SA-related genes and downregulates auxin-related genes, but surprisingly, an extremely resistant variety does the reverse. Studies of interactions with CPB revealed both plant responses (upregulation of stress, phytoalexin, PR, and PI genes) and insect adaptations (alterations in digestive proteases). One of the resistance mechanisms of plants is to produce proteinase inhibitors that inactivate digestive enzymes of insects that they require for feeding on host tissue. She showed that beetle larvae that were experienced on diets with plant proteinase inhibitors adapted their repertoire of digestive proteases, making them insensitive to proteinase inhibitors that they had already experienced (inhibitor-insensitive proteases). Importantly, field studies revealed that the response of these digestive proteins in PCB larvae was affected by PVY infection of host plants. Beetle larvae grew better on PVY infected plants than on non-infected plants and this was accompanied by differences in the expression of digestive enzymes and regulatory genes in the gut of beetle larvae, illustrating the diversity of mechanisms underlying plant-mediated microbe-insect interactions.

Vera Kuzina Poulsen addressed the value of ecometabolomics and ecogenomics approches to study plant-microbe-insect interactions, illustrated by biotic interactions in winter cress. In Denmark, winter cress occurs in two forms, one that is glabrous and resistant to flea beetles (G) but susceptible to an oomycete pathogen, the other being hairy and susceptible to flea beetles (P). She made a cross between a P and G genotype to produce segregating F2 lines and used a combination of bioassays, biochemistry, metabolomics, genomics, and phenomics to identify genes and metabolites that confer resistance. Four saponins were identified that were associated with resistance. Flea beetles that overcome plant resistance can detoxify the saponins by cleaving off sugars molecules. She sequenced the P and G type transcriptomes to search for candidate genes of classes involved in saponin biosynthesis, and developed molecular markers that discriminate between the G and P type. Two saponin QTLs nicely co-located with two resistance QTLs, both including a limited number of candidate genes. The flea beetle resistance QTL did not co-locate with QTL for resistances to the oomycete pathogen known from other crucifer species. She uderpinned the value of combined metabolomic and genomic approaches to understand mechanisms underlying trade-offs or synergies between pathogen and herbivore resistance.

As the general feeling at the end of the day was that the amount of time to discuss priority areas and follow-up activities during the last day of the workshop would be rather tight, the **evening discussion** was quickly focused around issues of applied aspects of plant-microbe-insect interactions and ideas for follow-up activities. (1) It was felt that for any funding activity, as researchers, we should make clear what is fundamentally novel about plant-microbe-insect interactions that distinguish this field from multitrophic interactions with microbes *or* herbivores. Partly this lies in the almost unlimited repertoire of changes that - particularly microbes - can bring about in their hosts, creating novel host phenotypes and innovations enabling novel functions that uniquely affect their interactions with insects. This has consequences for both ecology (unraveling “hidden players” in plant interactions with the biotic environment and assessing their community-wide implications, and their importance in a restoration, conservation, and invasion biology context) and for agriculture (combining the benefit of mutualistic microorganisms with their benefit in terms of enhanced pest resistance, attraction of biocontrol insects and reduced vector competence). (2) Opportunities for shaping a research network on plant-microbe-insect interactions were discussed. Given the uncertainty of calls for ESF Research Networking Programmes and EUROCORES themes in the near future it was decided to apply for an EU COST Action in the domain of Food and Agriculture.

Session 3 was devoted to ecological and evolutionary consequences of plant-microbe-insect interactions. Emphasis was on how plant interactions with microbes shape plant-associated populations, communities and dynamics of insects and vice versa.

Marcel Dicke addressed phenotypic variation in plants within a multitrophic context. Plants are often challenged by multiple attackers and the reponses of plants to herbivores strongly depend on whether they are already infected by pathogens or not, and vice versa. He showed that these responses are not always easy to predict. For instance, caterpillars of a cabbage specialist insect enhance systemic resistance against a biotrophic virus. This is surprising since resistance to the virus requires salicylic acid, and the caterpillar only induces jasmonic acid and ethylene. However, the ethylene sensitizes (primes) the leaves to respond faster to salicylic acid, enhancing virus resistance. Attack of plants by one enemy can also lead to alterations of the plant phenotype that affect indirect defenses of other attackers on the plant. For instance, whiteflies suppress the induction of volatile terpenes by spider mites on the same plant, that are involved in attraction of the spider mite’s natural enemies. He stressed the importance to study effects of multiple attack in a community context. The sequence of arrival of herbivores or pathogens, and their modification of the phenotype of the plant determine the plant’s suitability for ensuing herbivores and pathogens, and thereby structure the community of plant associated microbes, herbivores and natural enemies lateron in the season. Such community structuring effects, through alterations of plant phenotype by early attack, have thus far largely been neglected.

Bodil Ehlers addressed three-way interactions between thyme, legumes and rhizobia. She added an extra layer of complexity to plant-microbe-insect interactions by illustrating how the chemotype of one dominant plant species (shaped by pathogens, insects, or other aspects of the environment) not only alters the associated plant community, but also their biotic interactions. Thyme is a dominant plant species in carrigue vegetation in France. The main plant chemotypes differ in their profiles of monoterpenes that play a role in drought resistance, herbivore and pathogen defense, inhibition of germination of competing plant species, and effects on soil biota. She showed that thyme-associated plants exhibit an adaptive response (better germination and growth) to the home chemotype of thyme with which they co-occur. Populations also harbour more seed families with a positive growth response to the terpenes of their local chemotype than populations with another local chemotype. This strongly indicates selection by thyme on associated species. In fact, the chemistry of thyme translates into a specific “community phenotype”. She also showed that thyme chemistry affects species interactions across trophic levels. The terpenes leach into the soil, affecting nitrifiers and rhizobia, and thereby affecting interactions of thyme with co-occurring legumes. Specifically, the presence of thyme terpenes in soil shifts the genetic correlation between legume and rhizobia fitness from negative to positive, facilitating a more mutualistic interaction. The terpenes also affect interactions with pollinators and herbivores, making it interesting to study whether thyme chemotype can simultaneously structure the associated microbial and insect communities.

Tamara van Mölken addressed consequences of pathogen infection in plants for herbivore behaviour and plant fitness. Interactions between plant pathogens and herbivores are bound to occur in natural systems where both attack a plant. Winter cress is attacked both by theoomycete *Albugo candida* and the flea beetle *Phyllotreta nemorum*. In Denmark there are two plant types, one that is glabrous and resistant to flea beetles (G), the other being hairy and susceptible (P). Flea beetle resistance is mediated by specific saponins. Interestingly, the flea beetle-resistant G type is more susceptible to the fungal pathogen than the P type. She initiated a series of experiments to study whether pathogen infection affects flea beetle preference, consumption, and performance (through alterations of primary metabolism or induced defenses), and the effects of variation in pathogen and herbivore damage on plant fitness. She showed that effects of fungus and flea beetle are strongly dependent on each other. The herbivore increases disease symptoms and the pathogen increases larval damage; these effects depend on plant genotype.

Ayco Tack addressed how fungal-mediated interactions affect herbivore metacommunity dynamics. The metapopulation dynamics of both the phytopathogen *Podospaera plantaginis* and the lepidopteran herbivore *Melitaea cinxia* on the host plant *Plantago lanceolata* have been extensively studied for more than ten years on a series of islands between Sweden and Finland. Interactions between the pathogen and the insect can be observed at multiple spatial scales. At the individual level, pathogen infection prolongues larval development, and larvae are twice as likely to be parasitized by a hymenopteran parasitoid than uninfected plants. Analysis of metapopulation data show that a significant part of the variation in herbivore dynamics in these populations can in fact be explained by the presence or absence and history of pathogen infection in these islands. This shows the importance of pathogens in structuring insect communities across spatial scales. Similar observations were made for effects of mildew infections on colonization and extinction dynamics of leafminers, due to effects of mildew infection on the probability of leaf miners to be parasitized by their natural enemies.

Marcel van der Heijden addressed a community perspective of plant-microbe-insect interactions with the mycorrhizal symbiosis as an example. He addressed the role of AM fungi both from an ecological and agricultural perspective. Plant responses to AM fungi vary from positive to negative, the majority benefiting from the association. Interestingly, the majority of weeds is suppressed, while crop responses are variable. AM fungal diversity is higher in organic than in conventional farming systems. This is of significance since in nutrient poor environments, AM fungal diversity promotes productivity and plant diversity. This is due to complementarity effects; AM fungal mixtures increase yield more than any of the single AMF species. Legumes rely on the combination of rhizobia and AM fungi to successfully coexist with other plants. Here, total symbiont diversity (AM fungi and rhizobia) is expected to enhance productivity because they have complementary functions (providing phosphorus and nitrogen, respectively). Total symbiont diversity is also expected to enhance plant diversity (since different symbionts support different plant species) and to alter community structure, not only of plants but also of their associated insect community. He stressed the importance of symbiotic diversity and the need to understand its consequences for multitrophic interactions and food web ecology.

Session 4 was devoted to identify research priority areas and outlining follow-up activities. These are summarized in the next section.

3. Assessment of the results, contribution to the future direction of the field, outcome

What was learned, new research objectives

The workshop was inspiring to the participants and has resulted in several shared research initiatives between participants and joint follow-up activities. The overall conclusion of the workshop was that microbes bring about an almost unlimited range of alterations in their hosts, creating novel host phenotypes and innovations, enabling novel functions that uniquely affect their interactions with insects and vice versa. This has important consequences for both ecology (unraveling “hidden players” in plant interactions with the biotic environment and assessing the community wide implications, and their importance in a restoration, conservation and invasion biology context) and for agriculture (combining the benefit of mutualistic microorganisms with their benefit in terms of enhanced pest resistance, attraction of biocontrol insects and reduced vector competence).

The workshop identified number of areas that need to be explored to advance the field of plant-microbe interactions. (1) While for some groups of organisms (e.g. effects of mycorrhizae or rhizobia on plant-insect interactions) we begin to understand patterns and mechanisms, for others (notably endophytes) this is in its infancy, while they have enormous potential for affecting natural communities and exploitation in agriculture. Methods to reveal their identity and function are now being developed and should be fully exploited; (2) The outcome of interactions between plants, microbes and insects can vary from beneficial to harmful for the players involved, even within a single system (plasticity of outcome). The factors underlying such shifts even between mutualism and antagonism should be studied and understood in light of their consequences in natural communities and their potential use in food and agriculture; (3) Molecular studies begin to unravel how plants tailor their responses to microbes and insects. Phytohormones (JA, SA, ET) that are key players in induced defenses have been identified and reveal shared patterns in defense signaling triggered by particular guilds of insects and pathogens. But the role of other phytohormones, their interactions with the abiotic environment, the specificity arising from downstream activated defenses, the generality of these patterns across plant species, and their importance relative to other mechanisms underlying plant-microbe-insect interactions are still poorly understood and should be prioritized. (4) Studies are now beginning to reveal evidence for evolution and local adaptation in plant-microbe-insect interactions, but efforts are needed to discover who are the drivers in these processes. (5) Perhaps the most notable gap in our knowledge is understanding the significance of these three-way interactions in a community context, i.e. what are the consequences of interactions for the composition and dynamics of the associated community (rather than on individuals). These areas should be part of the future research agenda.

Concrete actions as follow up

We agreed on four concrete plants for follow-up activities.

1. Submit a preproposal for a EU-COST action in the Food and Agriculture domain on PMI interactions. As explained by the EU representative, Wolfgang Weisser, calls for ESF Research Networking Programmes and EUROCORES themes in the near future are uncertain due to the streamlining of activities of ESF in line with the needs of its Member Organizations. It was therefore decided to first apply for a EU COST Action in the domain of Food and Agriculture. It was decided to meet the first deadline for this on September 30, 2011. If the COST action gets funded, a next step will most likely be to initiate an application for an RTN at a later stage (2012/2013). Current status: a COST preproposal has been submitted.

2. Submit a proposal for a special journal issue on Plant-Microbe-Insect interactions. It was decided to aim for a Special Feature in a major ecological journal. Participants were asked to prepare proposals for contributions (title, authors, short summary) for evaluation by a small committee formed by some of the participants that will select contributions to be proposed to the Special Feature Editor of the journal. Current status: eight proposals have been evaluated. If the journal is interested, the aim should be to have MSs ready by April 2012.

3. Writing an application to organize a follow-up symposium on PMI interactions at the Universidad Internacional de Andalucia in 2012. These symposia have up to 17 invited speakers that will be fully funded and an open call for additional participants up to a maximum of 50 participants in total. The application will be initiated from CSIC, Granada. The deadline is end November, 2011. Additional funding will be applied for from the Spanish Ministry of Science and Education (deadline October 25, 2011). Other options are applying for organizing a Jac Monod conference or a symposium at the EMBO congress. Current status: additional funding has been applied for.

4. Setting up a website on Plant-Microbe-Insect research as a platform for the scientific community. Current status: the website has been published: see https://sites.google.com/site/plantmicrobeinsect/

4. Final programme

FINAL ROGRAMME

Thursday 18 August 2011

09.30-11.30	**Arrival and coffee**
11.30-11.50	**Welcome, aims and set-up of the Workshop** **Thure Hauser** (University of Copenhagen, DK) and **Arjen Biere** (Netherlands Institute of Ecology, NL)
11.50-12.10	**Presentation of the European Science Foundation (ESF)** **Wolfgang Weisser** (ESF Standing Committee for Life, Earth and Environmental Sciences - LESC)
12.10-	**Session 1: Three-way interactions between plants, microbes and insects: looking across systems from mutualists to antagonists**
12.10-12.35	**"The role of AM fungi in plant trophic interactions"** **Alison Bennett** (The James Hutton Institute, Dundee, UK)
12.35-13.00	**"The role of beneficial rhizobacteria in plant-insect interactions"** **Ana Pineda** (Wageningen University, NL)
13.00-14.00	*Lunch*
14.00-	**Session 1 ctd.**
14.00-14.25	**"Effects of soil microbes on plant-herbivore interactions"** **Anne Kempel** (University of Bern, CH)
14.25-14.50	**"Microbial mediated changes in flowering phenology and plant defense: implications for plant-pollinator and plant-aphid interactions"** **Eduardo de la Peña** (Ghent University, BE)
14.50-15.15	**"How plant-microbes influence host plant location by insect herbivores"** **Scott Johnson** (University of Western Sydney, Penrith, Australia)
15.15-15.45	*Coffee / tea break*
15.45-16.10	**"Endophytic fungi in grass species: are effects on herbivores and higher trophic levels predictable ?"** **Jochen Krauss** (University of Würzburg, DE)
16.10-16.35	**"Non-specialized endophytic fungi mediate plant-herbivorous insect interactions"** **Stefan Vidal** (Georg-August-University, Göttingen, DE)
16.35-16.45	*Ten-minute break*
16.45-17.10	**"Fungal endophytes of trees and how their beneficial potential may be studied and used as guards"** **Benedicte Albrectsen** (Umeå University, SE)
17.10-17.35	**"Plant green island phenotype induced by leafminers is mediated by bacterial symbionts"** **David Giron** (Institut de Recherche sur la Biologie de l'Insecte, Tours, FR)
17.45-19.00	*Dinner*
19.00-19.25	**"Effects of aphid symbionts on plant-insect interactions in a multitrophic context"** **Enric Frago** (Oxford University, UK)

19.25-21.30	**Discussion related to session 1; emergent topics collected during the day; exploration of topics in need of research focus**

Friday 19 August 2011

07.00-09.00	Breakfast
09.00-	**Session 2: Three-way interactions between plants, microbes and insects: insights from molecular biological studies and ecological implications**
09.00-09.25	**"Modulation of plant immunity by beneficial and parasitic organisms in Arabidopsis"** **Corné Pieterse** (Utrecht University, NL)
09.25-09.50	**"Impact of arbuscular mycorrhizas on aboveground plant-insect interactions: ecological and molecular aspects"** **Maria Pozo** (University of Granada, ES)
09.50-10.15	**"Geminiviruses inhibit jasmonate signaling, the hormones that mediate plant defense against insects"** **Eduardo Bejarano** (University of Malaga, ES)
10.15-10.45	*Coffee / Tea Break*
10.45-11.10	**"A plant virus can sense the presence of its insect vector on the infected host and immediately transmute into a transmissible morph"** **Stéphane Blanc** (INRA, Montpellier, FR)
11.10-11.35	**"Molecular basis of potato interaction with virus PVY and Colorado potato beetle"** **Kristina Gruden** (National Instutiute of Biology, Ljubljana, SI)
11.35-12.10	**"Ecometabolomics and ecogenomics of interactions between winter cress and flea beetles"** **Vera Kuzina Poulsen** (University of Copenhagen, DK)
12.10-12.35	**"Three-way interactions between Thyme, Medicago and Sinorhizobium"** **Bodil Kirstine Ehlers** (University of South Denmark, Odense, DK)
12.40-13.30	*Lunch*
13.30-17.30	**Walk though "De Blaauwe Kamer" (Rhine embankment reserve near Wageningen)**
17.45-19.00	*Dinner*
19.00-21.30	**Discussion related to session 2; emergent topics collected during the day; exploration of topics in need of research focus**

Saturday 20 August 2011

08.00-09.00	Breakfast
09.00-	**Session 3: Ecological and evolutionary consequences of three-way interactions between plants, microbes and insects**
09.00-09.25	**"Phenotypic variation in plants within a mutitrophic context"** **Marcel Dicke** (Wageningen University, NL)

09.25-09.50	**"Consequences of pathogen infection in plants for herbivore behaviour and plant fitness"** **Tamara van Mölken** (University of Copenhagen, DK)
09.50-10.15	**"Fungal-mediated interactions affect herbivore metacommunity dynamics"** **Ayco Tack** (University of Helsinki, FI)
10.15-10.45	*Coffee / Tea Break*
10.45-11.10	**"A community perspective of plant-microbe-insect interactions: the mycorrhizal symbiosis as an example"** **Marcel van der Heijden** (Agroscope RT, Zürich, CH)
11.10-	**Session 4: Outlining follow-up activities.**
11.10-12.30	**Part I: Summarizing cross-disciplinary areas that should be given research priority; outlining position papers; set-up of a joint paper that will summarize the conclusions from the meeting**
12.30-13.30	*Lunch*
13.30-15.00	**Part II: Steps to be taken to set up a research network for plant-microbe-insect interactions, ideas and outlines for joint reseach proposals, need for symposium series on plant-microbe-insect interactions; other networking activities; how to facilitate student and researchers exchange visits, etc.**
15.00	*End of Workshop and departure*

5. Final list of participants

1. **Benedicte Albrectsen**, Department of Plant Physiology, Umeå University, Umeå, Sweden
2. **Eduardo Bejarano**, Department of Cellular Biology, Genetics and Physiology, Area of Genetics, University of Malaga, Malaga, Spain
3. **Alison Bennett**, The James Hutton Institute, Dundee, Scotland, UK
4. **Arjen Biere (convener)**, Department of Terrestrial Ecology Netherlands Institute of Ecology (NIOO-KNAW), Wageningen, The Netherlands
5. **Stéphane Blanc**, Unité Mixte de Recherche Biologie et Génétique des Interactions Plante-Parasite, INRA-CIRAD-SupAgro, Montpellier, France
6. **Marcel Dicke**, Laboratory of Entomology, Wageningen University, Wageningen, The Netherlands
7. **Bodil Ehlers**, Institute of Biology, University of Southern Denmark, Odense, Denmark
8. **Enric Frago**, Department of Zoology, University of Oxford, Oxford, UK
9. **David Giron**, Institut de Recherche sur la Biologie de l'Insecte, Faculté des Sciences et Techniques, Tours, France
10. **Kristina Gruden**, Department of Biotechnology and Systems Biology, National Institute of Biology, Ljubljana, Slovenia
11. **Thure Hauser (co-convener)**, Department of Agriculture and Ecology, University of Copenhagen, Frederiksberg, Denmark
12. **Marcel van der Heijden**, Forschungsanstalt, Agroscope Reckenholz-Tänikon ART, Zürich, Switzerland
13. **Scott Johnson**, Hawkesbury Institute for the Environment, University of Western Sydney, Penrith, Australia
14. **Anne Kempel**, Institute of Plant Sciences, University of Bern, Bern, Switzerland
15. **Jochen Krauss**, Department of Animal Ecology and Tropical Biology, University of Würzburg, Würzburg, Germany
16. **Tamara van Mölken (co-convener),** Department of Agriculture and Ecology, University of Copenhagen, Frederiksberg, Denmark
17. **Eduardo de la Peña**, Terrestrial Ecology Unit, Department of Biology, Ghent University, Gent, Belgium
18. **Corné Pieterse**, Plant-Microbe Interactions, Department of Biology, Utrecht University, Utrecht, The Netherlands
19. **Ana Pineda**, Laboratory of Entomology, Wageningen University, Wageningen, The Netherlands
20. **Vera Kuzina Poulsen**, Department of Plant Biology & Biotechnology, Faculty of Life Sciences, University of Copenhagen, Frederiksberg, Denmark
21. **Maria Jose Pozo**, Soil Microbiology and Symbiotic Systems, Estación Experimental del Zaidín (CSIC), Granada, Spain
22. **Ayco Tack**, Metapopulation Research Group, Department of Biological and Environmental Sciences, University of Helsinki, Helsinki, Finland
23. **Stefan Vidal**, Department of Crop Sciences, Agricultural Entomology, Georg-August-University, Göttingen, Germany
24. **Wolfgang Weisser (ESF reresentative)**, Insitute of Ecology, Friedrich-Schiller-University, Jena, Germany

6. Statistical information on participants

The 23 invited participants originated from 11 European counties and one non-European country. The breakdown by country was as follows: DK (4), NL (4), DE (2), CH (2), ES (2), FR (2), UK (2), BE (1), FI (1), SE (1), SI (1), Australia (1). Participants were either invited directly, or recruited through an open call distributed via a general ecology and evolution directory. Despite country-selective recruitment from the resulting applications, countries from eastern parts of Europe were relatively underrepresented.

Breakdown of participants was not made by age but by carrier stage (years after PhD). There was an approximately equal distribution of participants at early stages of their carrier (0-6 yrs after PhD), more advanced stages (7-12 yrs after PhD), and senior scientists (13-24 yrs after PhD), represented by 8, 7 and 8 participants, respectively. Detailed breakdown of years since PhD (with number of participants in brackets): 0 (1x), 1 (2x), 2 (1x), 3 (1x), 4 (1x), 5 (1x), 6 (1x), 8 (1x), 9 (2x), 11 (1x), 12 (3x), 13 (1x), 17 (1x), 18 (2x), 20 (1x), 23 (3x).

The male/female repartition was approximately 60/40% (14 males vs. 9 females). Females were relatively over-represented among early (5/8) and more advanced carrier stages (4/7) and strongly under-represented among senior scientists (0/8).