

Chapter 6

Ecological cross-effects of induced plant responses towards herbivores and phytopathogenic fungi

Abstract

Plants are under continuous threat of being challenged by herbivorous insects and pathogenic micro-organisms. How plants respond to multiple antagonists that may co-occur has been investigated by researchers on the ecological, pharmacological, and genetic level. In this review we focus on recent studies of ecological cross-effects between phyllophagous herbivores and phytopathogenic fungi. Interactions between the two types of plant antagonists may be direct, plant-mediated or both. We present studies which describe a) potentially plant-mediated cross-effects but where direct effects cannot be excluded ('direct/plant-mediated effects'), b) plant-mediated cross-effects that are confined to the attacked plant part ('locally plant-mediated effects') and c) plant-mediated cross-effects on a systemic scale ('systemic effects'). Also, studies are addressed which attempted to directly link plant physiological changes induced by the herbivore and fungal attack with effects on the antagonists. Special attention will be given to spatial and temporal aspects of plant fungal infection and herbivore infestation.

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1. Introduction

Plants are constantly faced with the risk of being attacked by herbivorous insects or pathogenic fungi. Since insects and fungi are ubiquitously present in terrestrial systems, they may encounter each other on the same individual host plant or may try to exploit a host plant which has been altered by previous attack. The frequency and quality of interactions between

plants, herbivores, and fungi will depend on the specific biologies, lifecycles and population densities of the involved species (de Nooij et al. 1992). Co-occurring herbivores or fungi can interact directly. For example, fungi often utilize insects as vectors for their dispersal and some herbivores are known to feed facultatively upon fungus-infected plant tissue (Hammond & Hardy 1988). However, indirect plant-mediated interactions also take place. Both phytopathogens and herbivores are known to cause modifications in the allocation of primary plant metabolites and to trigger defensive plant responses (Karban & Baldwin 1997; Hammerschmidt & Nicholson 1999). As a consequence, fungal infection can change a host plant's suitability for herbivores and vice versa (Hatcher 1995).

To date, induced plant responses to herbivores and pathogens have predominantly been investigated in systems that focused on the relationship between plants and *either* herbivores *or* pathogens. Hence, in order to gain deeper insight into the complex interactions in nature, it is necessary to extend studies from two-component to three- or multi-component systems. Plant-fungus-herbivore interactions, and in particular induced plant responses against multiple enemies, have recently received considerable attention from different perspectives. Studies in this field span the range from taking a broader ecological view to research that focuses on the specificity of signalling pathways which are involved in plant defences against herbivores and pathogens (reviews: Hatcher 1995; Agrawal et al. 1999; Bostock 1999; Maleck & Dietrich 1999; Felton & Korth 2000; Bostock et al. 2001).

For example, several field studies of interactions between a leaf beetle and pathogenic fungi attacking *Rumex* species provided detailed knowledge on the ecological cross-effects between these plant antagonists when co-occurring (Hatcher et al. 1994a,b,c; 1995a,b; Hatcher 1995; Hatcher & Ayres 1997; Hatcher & Paul 2000). Other researchers concentrated on the specificity of elicited defence responses and their physiological and molecular base (Moran 1998; Fidantsef et al. 1999; Stout et al. 1999). In the fast moving area of signal transduction research two major pathways were defined to play an important role in plant resistance: the pathogen-associated salicylic acid pathway and the octadecanoid or jasmonate pathway which is generally associated with herbivore defence. However, there is no clear-cut dichotomy. Several studies show that pathogens and herbivores may stimulate the 'wrong' pathway (Karban & Kuc 1999). Cross-talks between signalling pathways indicate that induction of one pathway may have a synergistic (Bostock 1999) or attenuating effect on the

other pathway (Felton et al. 1999; Preston et al. 1999; Thaler 1999a). Additionally, transduction systems using wound signal messengers others than jasmonic acid or salicylic acid may also play a significant role (Sticher et al. 1997; Kahl et al. 2000; Bostock et al. 2001).

An important contribution to integrate ecological and molecular insights into a theoretical framework has been made by Paul and co-workers (Paul et al. 2000). Strong emphasis was placed on considering induced defences within the context of a general survival strategy of the plant. This broader view takes into account mechanisms that enable the plant not only to fend off but also to tolerate herbivore or fungal attack. Tolerance may be measured e.g. as shifts in the pattern of resource allocation within the plant, delayed senescence or enhanced photosynthesis in undamaged leaves. Selection for tolerance and regrowth should be particularly apparent in habitats where plants experience strong competition from other plants (Karban & Baldwin 1997). Although defence-related signal molecules like jasmonic acid can play a role in the growth and developmental processes of a plant, very little is known about how plants regulate the balance between defence and tolerance (Paul et al. 2000).

In contrast to a growing number of studies investigating molecular signalling pathways and their possible cross-talks, ecological studies on plant responses to multiple enemies are less numerous. Overviews considering ecological aspects of plant-mediated interactions between pathogens and herbivores were given by several authors, as e.g Hammond & Hardy (1988), de Nooij et al. (1992), Hatcher (1995), Hatcher & Ayres (1997). Here we intend to cover recent progress in the field of ecological cross-effects between fungal plant pathogens and herbivorous insects. Studies are reviewed here that tested the influence of plant pathogenic fungus infection on herbivore performance and/or behaviour. Conversely, experimental evidence for herbivore damage affecting fungal growth is considered. Because of space limitations, interactions involving endophytic fungi or insects as vectors for fungal diaspores, as well as mutualistic associations between fungi and herbivores, are not covered in this review. The bias towards phyllophagous insects and leaf-infecting fungi reflects the lack of studies carried out with antagonists attacking other plant parts such as stems or roots. Since results obtained in laboratory experiments are not necessarily relevant in the field, we differentiated between field and laboratory studies. Furthermore, special attention is paid to

spatial and temporal aspects of the interactions, as induced plant responses may vary greatly in this respect (Karban & Baldwin 1997).

2. Definition of effect categories

In this review we categorised the interactions between co-occurring plant pathogenic fungi and herbivorous insects as ‘direct/plant-mediated’, ‘locally plant-mediated’ or ‘systemic’. When herbivores feed on diseased leaves it may be difficult to distinguish the plant’s role from the impact that fungal structures may have on the herbivore and often no clear-cut distinction is made between direct and plant-mediated effects. Therefore, the term ‘direct/plant-mediated effects’ was introduced. This term was used in cases where - apart from induced plant responses - the fungus itself could also have had an immediate impact on the herbivore due to contact or ingestion of fungal structures together with the surrounding leaf tissue. Also, herbivore activity may have a direct effect on the fungus, e.g. by changing the leaf surface structure, a plant trait important for some fungi for host recognition. We decided to include studies on direct/plant-mediated effects i) since induced plant responses could have played a role in the interaction and ii) because we would like to show that actually only few studies exist that have demonstrated truly plant-mediated effects by excluding direct factors in the experiments. However, as mentioned above, studies on mycophagy or on insects functioning as vectors for fungi are not addressed here. In addition we defined ‘locally plant-mediated effects’ as plant-mediated effects on a local scale i.e. on the scale of the herbivore-damaged or fungus-infected leaf. These effects are evident if feeding upon a fungus-infected leaf, without detectable fungal structures, affects the performance or behaviour of the herbivore or if any direct influence of herbivore activity on the fungus can be ruled out. Systemic effects are evident if symptom-free leaves of an attacked plant confer enhanced resistance or susceptibility.

Definitions:

- **Direct/plant-mediated effect** = Effect on antagonist 2 results from the physical presence or activity of antagonist 1 *and/or* from plant responses induced by antagonist 1.
- **Locally plant-mediated effect** = Effect on antagonist 2 results exclusively from plant responses induced by antagonist 1. Both antagonists are or were present on the same morphological entity of the plant (typically: leaf).
- **Systemic effect** = Effect on antagonist 2 results exclusively from plant responses induced by antagonist 1. Each antagonist is or was active on a different morphological entity of the plant.

3. Effects of fungus-induced plant responses on herbivores Table 2

Host plants infected by pathogenic fungi represent a complex feeding niche for herbivorous insects. Diseased plants differ morphologically and physiologically from healthy individuals. Leaf chlorosis and necrosis or the formation of ‘green islands’, which are spots of photosynthetically active tissue surrounded by chlorotic areas, as well as wilted leaves, give plants an altered appearance (Hammond & Hardy 1988). The fungal pathogen itself produces nutrients and secondary metabolites, such as fungal toxins. Furthermore, the fungus may induce changes of primary and secondary plant metabolites (Ayres 1992; Lucas 1998). Consequently, insects may display an untypical host selection behaviour on encounter with infected plants. Herbivores can be especially attracted to diseased plants or they may be deterred when compared with healthy plants. But not only the behaviour of the herbivorous insect may be affected, the plant pathogenic fungus may also influence herbivore performance. Both beneficial or detrimental impacts on the insect’s performance are known.

3.1. Effects of fungus-induced plant responses on herbivore behaviour

The most striking differences between herbivores and pathogens are the herbivore’s physiological autonomy and its mobility (Baldwin & Preston 1999). Insects in search for a suitable host may be able to recognize a suboptimal plant, e.g. one that is infected by a pathogenic fungus, and may consequently avoid this host. In particular, this should be true for the adult stage as it is often more mobile than the larvae. Therefore, if feeding on a fungus-infected plant can have adverse effects on the insect or its progeny, avoidance behaviour should be a good strategy for a herbivore to increase its fitness.

3.1.1 Direct/plant-mediated and locally plant-mediated effects of fungus-induced plant responses on herbivore behaviour

Laboratory experiments. Fungal infection of plants has often been observed to deter herbivores from feeding or ovipositing where herbivores encountered infected tissue. As outlined above (see 2.), most of the studies available did not discriminate between direct and plant-mediated effects of fungal infection on herbivores

Recently, Simon & Hilker (unpublished data) tried to separate possible direct effects of fungal infection from locally plant-mediated ones (Table 1). The effects of infection of the willow *Salix x cuspidata* by the rust *Melampsora allii-fragilis* on the willow leaf beetle *Plagioderma versicolora* have been studied. It was possible to infect willow leaves in a way that only one half of a leaf had visible rust symptoms whereas the other half remained symptom-free. By offering the symptom-free half of an infected leaf to *P. versicolora*, locally plant-mediated effects could be studied, while offering the rust infected leaf half allowed to observe direct and/or plant-mediated effects. At 12 dpi, adult *P. versicolora* preferred to feed upon the symptom-free half of the leaf to feeding upon the directly infected half with visible rust symptoms. However, at 12 dpi symptom-free halves of willow leaves were not consumed significantly less than healthy leaves of an uninfected plant. Thus, no locally plant-mediated effects of rust infection towards feeding of the leaf beetle were detected, while the avoidance of leaf halves with rust symptoms at 12 dpi was probably due to direct effects of the fungus.

Table 1 Triple-choice test assessing the effect of direct/plant-mediated and locally plant-mediated rust infection [12 dpi] by *Melampsora allii-fragilis* on the feeding preference of adult *Plagioderma versicolora*. Different letters indicate significant differences. Friedman-ANOVA and Wilcoxon-Wilcoxon-test ($p < 0.01$). $N = 40$

	Uninfected leaf	Rust-infected leaf half	Symptom-free leaf half
Mean leaf area consumed [cm ²]	0.41	0.08	0.34
Standard deviation	0.27	0.16	0.21
Significance	a	b	a

Infection of plant tissue by rust fungi is well-known to affect herbivore behaviour (Table 2). Certainly one of the most intensively studied tripartite systems consists of the leaf beetle *Gastrophysa viridula*, the biotrophic rust fungus *Uromyces rumicis*, and their common

host plants *Rumex obtusifolius* and *R. crispus*. In laboratory choice tests rust infection of *R. obtusifolius* had a negative direct/plant-mediated effect on the beetles' oviposition behaviour. Leaves on which *U. rumicis* had grown for eight days were laden with about half as many eggs as healthy controls. Adult *G. viridula* also preferred to feed on healthy leaves (Hatcher et al. 1994c). Deterrent direct/plant-mediated effects of fungus-infected plants on herbivores were also reported from other natural plant-rust-herbivore systems. Infection of the musk thistle *Carduus thoermeri* with the rust fungus *Puccinia carduorum*, a biocontrol agent introduced to the United States, led adults of *Cassida rubiginosa* (Chrysomelidae) and *Trichosirocalus horridus* (Curculionidae) to consume more healthy than rust-infected leaf tissue when given the choice. But the number of eggs laid did not differ between healthy and diseased plants. On infected leaves, feeding and oviposition were confined largely to pustule-free areas (Kok et al. 1996). Also last instar larvae of the lepidopteran species *Tyria jacobaeae* fed more on healthy leaves of the host plants *Senecio vulgaris* and *Tussilago farfara* than on leaves of both hosts on which the rust fungus *Coleosporium tussilaginis* sporulated (ca. 14 dpi) (Tinney et al. 1998).

Negative direct/plant-mediated effects of a necrotrophic fungus on a leaf beetle were demonstrated in an agricultural system. Chinese cabbage leaves, with 10-15% of the leaf area infected by *Alternaria brassicae* (5 dpi), were avoided by females of the chrysomelid beetle *Phaedon cochleariae*. Leaf consumption and oviposition was higher on comparable healthy leaves (Rostás & Hilker in press).

Herbivores may also prefer plant tissues infected by pathogenic fungi, although reports on such interactions are less numerous (but see Hammond & Hardy 1988, Hatcher 1995). For example, the leaf beetle *Diabrotica undecimpunctata* removed a larger portion from cucumber leaf disks if these were infected with the necrotrophic fungus *Cladosporium orbiculare* for 3 days (Moran 1998). Second-instar larvae of *P. cochleariae* were found to consume more from Chinese cabbage leaves infected with *A. brassicae* than from healthy ones. No-choice assays showed that this was not due to compensatory feeding. Interestingly, larval discrimination between healthy and infected cabbage leaves contrasted with adult behaviour. Adults avoided *A. brassicae*-infected leaves (Rostás & Hilker in press).

Recently, a mutualistic relationship between a herbivore and a biotrophic fungus has been demonstrated for the first time. The weevil *Apion onopordi* deposits its eggs preferably in rust-infected shoots of the thistle *Cirsium arvense* during a period of 72 h, as the beetle's offspring develop better in such hosts (see 3.2.1). However, when ovipositing into a healthy thistle shoot, female weevils transmit the rust fungus *Puccinia punctiformis* to the base of the shoot. This is a prerequisite for a systemic infection of the roots (Friedli & Bacher 2001).

Field experiments. In the *Rumex*-rust-*Gastrophysa* system, where laboratory choice tests had given evidence for deterrent direct/plant-mediated effects of fungal infection on adult *G. viridula*, such effects were partly also found in the field (Hatcher et al. 1994c). The beetles laid more eggs on healthy than on diseased *R. obtusifolius*, but consumed similar quantities of leaf tissue. No preferences were found in this regard on diseased or healthy *R. crispus*, thus confirming the outcome of the laboratory studies.

A different approach in the methodology of evaluating effects of plant disease on herbivore behaviour was taken by assessing the insect and fungus community structure in the field. The quantitative density of three chrysomelid cucumber beetles, including *Diabrotica undecimpunctata howardi*, *D. virgifera virgifera*, and *Acalymma vittatum* was found to correlate negatively with the presence of powdery mildew *Erysiphe cichoracearum* on leaves of squash plants *Cucurbita pepo* x *texana* (Moran & Schultz 1998). A feeding type specificity becomes obvious when looking on other fungus - squash plant - herbivore interactions. While cucumber leaf beetles appear to be negatively affected by intense *Erysiphe* infection of the *Cucurbita* host plants, no interactions were found between this plant-fungus complex and two sucking herbivores (*Aphis gossypii*, *Anasa tristis*). On the other hand, densities of *D. undecimpunctata howardii*, *A. vittatum*, and *A. gossypii* did not differ on another host plant, *Cucumis sativus*, two weeks after being inoculated with the scab fungus *Cladosporium cucumerinum* (Moran & Schultz 1998).

In a noteworthy study, Kluth and co-workers (2001) had assessed the impact of natural rust (*P. punctiformis*) infection of the thistle *C. arvense* on 47 ecto- and endophagous herbivores. Fungal infection had a differential impact on insects. Most ectophages from the orders Coleoptera, Heteroptera, and Homoptera were equally abundant on healthy and diseased plants. However, aphids and some beetle species preferred infected plants. Endophagous

herbivores, on the other hand, were more abundant on healthy thistles with larvae of the weevil genus *Apion* being the exception.

3.1.2 Systemic effects of fungus-induced plant responses on herbivore behaviour

There is little evidence that fungal infection affects the behaviour of herbivorous insects on a systemic scale. However, seedlings of the naturalised weed *Brassica rapa* which had been infected by the fungal pathogen *Leptosphaeria maculans* for 1 day, showed enhanced resistance against the diamondback moth *Plutella xylostella*. Larvae fed significantly less on non-symptomatic cotyledons of *B. rapa* from infected plants when compared to healthy, uninfected plants. This study also showed that the resistance effect was dependent on the level of secondary compounds in the plant. At highest levels of glucosinolates that were detected in locally and systemically infected cotyledons, plants were most resistant to herbivory (Siemens & Mitchell-Olds 1996).

Adults of the willow leaf beetle *P. versicolora* feeding on willow leaves are able to discriminate not only between leaves from healthy trees and symptom-free leaves of a rust-infected plant, but also between systemic leaves from different positions. Simon & Hilker (unpublished data) demonstrated that systemic leaves close to the infection site were avoided by feeding beetles [at 12 dpi], while leaves further up or down of the infection site were treated like healthy leaves by the insect.

No systemic effects of scab fungus infection in cucumber have been found on the feeding behaviour of cucumber leaf beetles. The herbivores did not remove differing areas from systemic or healthy leaves when tested at intervals of 3 to 6 dpi (Moran 1998). Likewise, neither adults nor larvae of the mustard leaf beetle *P. cochleariae* showed any preference for healthy leaves of Chinese cabbage *B. rapa* ssp. *pekinensis* or symptom-free leaves from plants infected by *A. brassicae* [5 dpi] (Rostás & Hilker in press).

3.2. Effects of fungus-induced plant responses on herbivore performance

Plant fungal infection may have an impact on various performance parameters of herbivorous insects. The most pronounced effects were found when insects fed upon infected plant tissue, but also some locally plant-mediated and systemic effects have been reported (Table 2).

3.2.1 Direct/plant-mediated and locally plant-mediated effects of fungus infection on herbivore performance

Laboratory experiments. Adults of the chrysomelid *G. viridula* may be deterred by rust-infected dock plants (see 3.1.1), and thus opt for a more suitable host. However, if females oviposit on rust-infected leaves their progeny suffer greater larval mortality. Also, larvae feeding on infected leaves have lower relative growth rates (RGR) and convert ingested food less efficiently, while resulting adults show reduced fecundity (Hatcher et al. 1994c). Likewise, the performance of the chrysomelid *P. versicolora* was detrimentally affected when fed rust-infected willow leaves [dpi]. Larval mortality was higher and developmental time was prolonged when larvae were reared on diseased leaves (Simon & Hilker, unpublished results).

In another natural system the effects of feeding on infected tissue were only apparent when host plants of low suitability were offered. Caterpillars of the cinnabar moth suffered from fungal presence when feeding on fungus-infected leaves of the suboptimal host plant *Tussilago farfara*. However, when feeding on the more suitable host plant *Senecio vulgaris*, no adverse effects of fungal infection on the caterpillar were found. Thus, not the phytopathogenic infection *per se* was able to affect the herbivore, but the combination of low host plant suitability and plant pathogen infection had significant detrimental effects (Tinney et al. 1998). No differences in the sizes and weights of larvae of the beetles *C. rubiginosa* and *T. horridus* were noted when reared on rust-infected thistles *C. thoermeri* (Kok et al. 1996).

A case of enhanced susceptibility of fungus-infected plants towards herbivores was found in cucumber leaves bearing necrotic symptoms of the scab fungus *C. cucumerinum*. About 50 % more offspring was produced by melon aphids on these leaves than on healthy foliage. However, this positive effect of fungal infection on the herbivore was observed only when the necrotic area did not exceed 50 % of the total leaf area. On very severely infected leaves the fecundity of *A. gossypii* was reduced (Moran 1998).

A very thorough investigation to explicitly assess locally plant-mediated responses and their specificity on herbivore performance has been carried out by Stout and co-workers (Stout et al. 1999). Plant responses elicited by pathogens and herbivores and their subsequent cross-effects on either plant antagonist type were assessed. This was done by confining the terminal leaflets of tomato plants to either bacterial (*Pseudomonas syringae* pv. tomato) or fungal (*Phytophthora infestans*) treatment for a period of 120 h. Thereafter, the remaining untreated leaflets of the induced leaves were fed to the noctuids *Helicoverpa zea* and *Spodoptera exigua* (only *P. infestans*). To avoid confusion it is noteworthy that the authors themselves categorised the treatment as systemic. *H. zea* feeding on leaflets of *P. syringae*-infected plants for 24 h had lower relative growth rates (RGR). However, the infection by the fungus *P. infestans* had no plant-mediated effect on the suitability of tomato leaves to both noctuids. In this study, only RGR have been measured and the exposure to the leaflets was short. Thus, it remains unknown if the fungus *P. infestans* has any significant impact on other performance parameters of the herbivores, such as mortality or fecundity of the resulting adults.

On a local scale fungus-induced, plant-mediated resistance towards herbivores was also found in Chinese cabbage, *B. rapa* ssp. *pekinensis* (Rostás & Hilker in press). Leaves of Chinese cabbage which had been infected by the causal agent of the black spot disease, the fungus *A. brassicae*, for 5 days had a detrimental impact on larvae of the mustard leaf beetle *P. cochleariae*. The speed of larval growth was reduced and pupae weighed less compared to larvae fed on healthy leaves. No direct impact on larval mortality was found, however, slow-growing larvae on pathogen-affected leaves showed increased susceptibility towards the entomopathogenic fungus *Metarhizium anisopliae*. The mortality of slow-growing larvae was twice as high as the mortality of larvae on healthy Chinese cabbage leaves (Rostás & Hilker submitted, a).

Field experiments. The direct/plant-mediated effects of naturally fungus-infected birch leaves on herbivore development were observed by Lappalainen and co-workers (Lappalainen et al. 1995) at the end of a vegetation period. Larvae of the autumn moth *Epirrita autumnata* feeding on birches infected with the rust *Melampsoridium betulinum* had differing pupal weights, depending on the severity of the disease. The highest pupal weights were attained by larvae which had developed on leaves with a low infection rate of ca. 1 % of the leaf area.

Infection of 3 % or 9 % of the leaf area resulted in significantly decreased pupal weights. First-instar mortality of *G. viridula* larvae was almost 90 % on rust infected *R. obtusifolius* compared to 23 % on healthy plants. This was due to the progressing leaf senescens caused by the fungus (Hatcher 1994c).

In field-collected thistles *C. arvensis*, the newly hatched adults of the weevil *A. onopordi* were twice as heavy if they had spent their larval stage in plants that were infected by the rust *P. punctiformis* compared to weevils that had developed in healthy plants. The larger weevils from infected plants also produced more and larger eggs (Friedli & Bacher 2001).

3.2.2 Systemic effects of fungus-induced plant responses on herbivore performance

Some attempts have been made to assess whether fungal infection restricted to some leaves of a plant affects host suitability of adjacent symptom-free leaves to insect herbivores. No evidence was detected for systemic effects of rust infection of dock plants on the performance of the leaf beetle *G. viridula* (Hatcher et al. 1994c). Neither were systemic effects of fungal infection on herbivore performance found in the tripartite system Chinese cabbage – fungus *A. brassicae* – mustard leaf beetle *P. cochleariae* (Rostás & Hilker in press). Likewise, no effects of the anthracnose fungus *Colletotrichum lagenarium* on the suitability of systemic cucumber leaves towards the herbivore performance of *Tetranychus urticae*, *Spodoptera frugiperda*, and *Aphis gossypii* were found (Ajlan & Potter 1991).

3.3 Spatial and temporal scale of fungus-induced plant responses on herbivore performance

3.3.1 Spatial aspects

At the current stage of research, direct/plant-mediated and locally plant-mediated effects of fungus-induced plant responses on herbivores are more obvious than systemic ones. The most drastic changes in plant morphology and physiology caused by fungal pathogens occur where hyphae border or enter plant cells and also in the plant tissue surrounding the infection site. Herbivores may encounter plant tissue of deteriorated quality as a result of nutrient depletion caused by the fungus, the activity of phytotoxins and other metabolites released by the

pathogen (de Nooij et al. 1992). Furthermore, the plant may show strong local responses to fungal attack by a hypersensitive reaction, cell wall lignification or the release of phytoalexins at the infection site (Kombrink & Somssich 1995; Kombrink & Schmelzer 2001). While several herbivorous insects may be negatively affected by such fungus-induced plant responses, others may profit from predigestion of complex carbohydrates by the fungus or by ingesting the fungus itself which may contain important nutrients like sterols (see 3.1 and 3.2) (Carruther et al. 1986, Mondy & Corio-Costet 2000).

Systemic responses of a plant to fungal attack range from structural alterations of plant cell walls, even at sites distant from the infection tissue, to production of pathogenesis related (= PR) proteins (Sticher et al. 1997; Hammerschmidt 1999). Such morphological and physiological changes in plant quality are expected to affect also herbivores, even though evidence for this is scarce (see 3.2 and 5).

It is well-known that a plant's susceptibility towards fungal infection varies within a plant as well as during its ontogenesis (for references see Heil 2001). For example, sporulation is known to decrease markedly with ascending leaf position (e.g. Boyle & Aust 1997). Herbivores might try (and succeed) to escape from the detrimental effects of fungus-induced systemic plant responses. They may choose leaves which have not been altered by the fungus with respect to their nutritional value or which have even improved in their suitability to herbivore consumption. However, also in healthy, uninfected plants the susceptibility of leaves to herbivores varies with leaf position (Schoonhoven et al. 1998). Thus, to detect the effects of fungally induced systemic plant responses on herbivores, further studies are necessary which focus on how herbivores are affected by leaves from different positions up and down the infection site and which compare the suitability of these leaves with leaves of the same positions from uninfected control plants.

3.3.2 Temporal aspects

The impact of a diseased plant on herbivore behaviour or its performance depends also on the time frame the investigator chooses when studying tripartite interaction. The temporal aspect is important in two ways. On the one hand, it determines the severity of damage a plant

experiences as the fungus will grow and spread within the tissue once it has established itself. On the other hand, it determines the resistance state of the plant because certain defences need a certain length of time to be mounted (Kombrink & Somssich 1995). The level of damage can influence the strength and type of the induced resistance mechanisms of a plant. The strength of resistance usually increases as the level of damage increases (Karban & Baldwin 1997). Furthermore, the chosen time frame determines the developmental state of the fungus and thus the physiological state of the infected tissue. For example, plant tissue infected by a rust fungus varies in its content of primary metabolites depending on whether sporulation had occurred or not (Wagner & Boyle 1995).

The importance of the lag time between fungal infection and subsequent herbivore attack has been shown in a study by Moran (1998) which revealed that fungus-infected cucumber leaves were significantly preferred for feeding by adult spotted cucumber leaf beetles at 3 dpi, but not at 4 – 6 dpi. Similarly, adults of the leaf beetle *P. versicolora* were deterred from feeding on infected willow leaves if the rust fungus *M. allii-fragilis* was allowed to grow for 16 days. No effects were observed at 8 or 12 dpi (Simon & Hilker, unpublished data). This outcome was due to locally plant-mediated effects. However, when direct/plant-mediated effects were assessed there was no difference in the preference for willow leaves at 8, 12 or 16 dpi.

3.4 Specificity of fungus-induced plant responses on herbivores

Plants are apparently able to defend against fungal pathogens by common basic strategies comprising very rapid, localised (e.g. hypersensitive response) and delayed, systemic (e.g. PR-proteins) mechanisms (Kombrinck & Schmelzer 2001). Nevertheless, species-specific differences can occur which may be due to differential recognition and triggering of complex signal cascades and gene products. The highest specificity is to be expected at the level of pathogen recognition by the plant, where gene-for-gene interactions may take place (Lucas 1998; Karban & Kuc 1999; Paul et al. 2000). This specificity may be conserved if distinct signal transduction pathways result in different defensive reactions or it may be lost as the transduction pathways merge downstream, thus resulting in the same chemical defence products (Stout & Bostock 1999). If different pathogens evoke different plant responses, these may also vary in their impacts on herbivores. Experimental data on this issue are scarce but

for example, caterpillars of *T. jacobaeae* were deterred from feeding on plants infected by the fungus *C. tussilaginis* but not from plants infected by *P. lagenophorae* (Tinney et al. 1998). However, this could also be the result of a direct interaction between fungi and insect. Furthermore, resistance of fungus-infected plants against the herbivore could depend on the mode of insect feeding. Differences in the densities of leaf-chewing and sap-sucking insects were found on cucurbits infected with a powdery mildew fungus (Moran & Schultz 1998). Cucumber beetles seemed to avoid infected plants while melon aphids were not affected by the presence of mildew. However, the authors point out that it remains unclear whether these interactions were mediated by chemical defences or the result of fungal effects as e.g. physical obstruction by mildew mycelia or spores. Furthermore, Kluth and co-workers (Kluth et al. 2001) observed that three specialised aphid species preferred rust-infected thistles, while most other leaf-chewing insects did not differ between healthy and diseased plants. Higher concentrations of amino acids in the phloem sap, as a result of fungally induced senescence-like changes within the leaf, were thought to be responsible for the greater numbers of aphids found. Thus, leaving aside direct effects by the fungus, the outcome of such tripartite interactions may depend on (a) the species specific response of the plant towards the pathogenic fungi, (b) the way pathogen-induced pathways interact with the (specific) responses induced by herbivore damage, (c) the chemical changes that result as a sum from these interactions and their feeding type specific effects on the herbivore and finally (d) the species specific temporal and spatial aspects as outlined above. Clearly, more experiments are needed that assess the plant-mediated impact of various fungal pathogens on the same herbivore.

4. Effects of herbivore-induced plant responses on fungal phytopathogens Table 3

Herbivore attack is well-known to induce a wide range of plant defensive responses (Karban & Baldwin 1997). These may act directly against herbivores e.g. by the production of toxic secondary components or defensive proteins that impair the digestibility of plant nutrients (Baldwin & Preston 1999). Indirect defensive plant responses against herbivores act by the emission of feeding- or oviposition-induced volatiles that attract antagonists of the herbivores (Dicke 2000 and references therein; Meiners & Hilker 1997, 2000; Hilker & Meiners in

press). While feeding-induced plant responses have been shown to affect fungal phytopathogens (Table 3), oviposition-induced responses have not been studied so far for their effects on phytopathogens.

4.1 Direct/plant-mediated and locally plant-mediated effects of plant damage by herbivores on fungal phytopathogens

Laboratory experiments. Several laboratory experiments have shown direct/plant-mediated and locally plant-mediated effects of herbivore feeding on the growth of phytopathogenic fungi. Direct/plant-mediated effects were demonstrated in the watermelon *Citrullus lanatus* infested by *Thrips tabaci* or *Aphis gossypii* two days prior to fungal infection. Watermelon showed less severe infection by the necrotrophic fungus *Colletotrichum orbiculare* than watermelon without thrips infestation (Russo et al. 1997). Likewise, Hatcher and co-workers (Hatcher 1994b) showed that feeding of 2nd instar larvae of the chrysomelid *G. viridula* upon dock plants *R. crispus* and *R. obtusifolius* for one day could significantly reduce pustule density of the rust fungus *U. rumicis* both directly at the damaged leaf parts (1, 3 and 7 days post-feeding) and at the undamaged parts of a grazed leaf (locally plant-mediated effect) (3 and 7 days post-feeding). Artificial damage of leaves had a similar impact on the fungus as herbivory. The attenuated fungal infection on (feeding) damaged dock plants was mainly caused by a negative effect on the prehaustorial stages of *U. rumicis* (Hatcher et al. 1994b, 1995a). Locally plant-mediated effects were also observed by Stout and co-workers (Stout et al. 1999). Inoculation of tomato plants with the bacterium *P. syringae* pv. *tomato* did not only induce resistance against larvae of the moth *H. zea* (see chapter 3.2.1), but conversely, two days of larval feeding also reduced the number of lesions caused by the pathogen.

However, herbivory prior to fungal inoculation of a plant does not always result in induced resistance against fungi. For example, feeding by the willow leaf beetle *P. versicolora* upon potted plants of the willow hybrid *S. x cuspidata* for 3 days had no influence on the number of pustules produced by the biotrophic rust fungus *M. allii-fragilis* on feeding-damaged leaves. The results of these greenhouse experiments were confirmed in the field, regardless whether feeding damage was caused by three larvae, ten larvae, or four adults, respectively (Simon & Hilker, unpublished data). Similarly, growth of *A. brassicae* on Chinese cabbage was not affected by feeding of larvae of the mustard leaf beetle *P. cochleariae* on the same leaves

(Rostás & Hilker in press). This study also assessed exclusively locally plant-mediated effects as herbivore treatment and fungal infection were carried out on different sites of the same leaf.

Enhanced susceptibility towards fungal infection was found in two poplar cultivars: the removal of 10% or 40% of the leaf area by the leaf beetle *Chrysomela scripta* or infestation by a spider mite (*Tetranychus* spp.) had a positive impact on the leaf spot pathogen *Septoria musiva* (Klepzig et al. 1997).

Field studies. Direct/plant-mediated effects of herbivory on fungal infection were found in field studies correlating the presence and absence of herbivores and fungi. For example, herbivory on squash plant was not found to affect the area covered by the fungus *E. cichoracearum* (Moran & Schultz 1998). However, disease severity of *Diaporthe phaseolorum* stem canker was increased on two different varieties of soybean infested with the alfalfa hopper *Spissistilus festinus* (Padgett et al. 1994).

4.2 Systemic effects of plant damage by herbivores on fungal phytopathogens

Laboratory experiments. As mentioned above, feeding of *G. viridula* on dock weed restricted the pustule density of rust on herbivore-damaged leaves already 1 day post-feeding. Seven days after herbivory had ceased, infection was also reduced on adjacent non-symptomatic leaves (Hatcher et al. 1994b). This suggests that a herbivore-induced signal spread systemically from the site of damage to distant parts of the plant.

No such systemic effects of herbivory on fungal infection were observed in the tripartite system consisting of Chinese cabbage, the necrotrophic fungus *A. brassicae*, and the mustard leaf beetle *P. cochleariae*. Inoculation of the fungus was carried out at 3 or 8 days after herbivory had ceased or without time lag (Rostás & Hilker in press). The failure to find any effects of herbivory on fungal growth could result from selection for faster growth and increased yield in domesticated plants at the expense of a strong defence system (Massei & Hartley 2000).

In contrast, grazing of *P. versicolora* on leaves of the willow hybrid *S. x cuspidata* resulted in a significant increase in the number of rust pustules on leaves adjacent to the feeding-

damaged ones (Simon & Hilker, unpublished results). This is the only tripartite interaction, so far, that revealed induced systemic susceptibility to subsequent fungal infection already one day after feeding had stopped.

Field experiments. Hatcher & Paul (2000) demonstrated that systemic inhibition of fungal growth on dock plants damaged by feeding of the leaf beetle *G. viridula* was not confined to controlled environments. Herbivore-damaged dock plants were more resistant to natural infection of the biotrophic rust *U. rumicis* (in autumn) and the hemibiotroph *Venturia rumicis* (in spring and autumn). Effects on the necrotrophic fungus *Ramularia rubella* were either not significant or not relevant (i.e. infection rate was too low in both treatments). Field studies of soybean *Glycine max* also revealed induced fungal resistance when plants were defoliated by the soybean looper *Pseudoplusia includens*. Fourteen days of feeding by 300 larvae caused a reduction in the number and area of stem cankers by the fungus *Diaporthe phaseolorum*. Likewise, 11 days of feeding reduced the number of perithecia and increased the distance of perithecia above the soil line of the red crown rot *Calonectria crotalariae* (Padgett et al. 1994).

4.3 Spatial and temporal scale of effects of herbivore inflicted plant damage on fungal phytopathogens

Spatial aspects. On a local scale, wounding of plants by herbivores disrupts the plant surface and thus, provides entrance for numerous phytopathogens. Fungi are able to penetrate the intact plant surface, however, changing the plant surface structure by herbivore feeding might impair fungal growth since fungi recognize their host by leaf surface structures (e.g. Staples et al. 1985). Feeding damage on plant tissue may interfere with the formation of fungal appressoria which are known to be induced by a variety of plant cues such as plant volatiles, potassium ions, sugars, and carbon dioxide concentrations (for references see Hatcher et al. 1995a). All these cues may be altered by herbivore feeding. Furthermore, herbivory might enhance humidity at the feeding site by disrupting plant cells and by the deposition of moist faecal matter. Enhanced humidity might improve the microhabitat and thus the developmental conditions for fungal growth. The effect of herbivore feeding on fungal plant pathogens on a local scale, i.e. at the feeding-damaged leaf, is suggested to be the sum of all changes, which may be both beneficial and detrimental for the fungus.

In addition to such direct effects of herbivore feeding, defensive plant responses caused by the herbivore and probably intended to fend off the herbivore might be the reason for the impact of feeding damage on fungi on a local and systemic scale (Felton & Korth, 2000; Paul et al. 2000). One might expect stronger or faster effects of feeding damage on fungi near the damaged site compared to more distant, i.e. systemic areas (Hatcher et al. 1994b). Irrespective of the feeding intensity, in almost all systems tested herbivory led to local or systemic induced resistance. However, in two studies including salicaceous trees, herbivore feeding was found to increase fungal susceptibility either locally (Klepzig et al. 1997) or systemically (Simon & Hilker unpublished data, compare 4.2).

Temporal aspects. The studies on the effects of herbivore feeding on fungal growth reviewed here considered time periods of days to a few weeks. Herbivore feeding is known to be able to induce plant defensive responses towards subsequent feeding after hours or days (short term induction), but also in the season following herbivore damage (delayed induced responses) (Haukioja 1990a, b; Karban & Baldwin 1997). Yet, whether herbivory also affects fungal growth in the subsequent season has not been investigated so far to our knowledge.

The lag time between herbivore feeding and fungal attack has been shown to be crucial for detecting systemic effects. For example, while inoculation of dock plants with rust 3 days after herbivore feeding did not reveal any systemic effects of feeding damage on the pustule density, fungal attack seven days after feeding damage led to significantly reduced fungal growth on leaves adjacent to those that were damaged (Hatcher et al. 1994b).

The influence of feeding duration on phytopathogenic fungi has hardly been studied so far. In the tripartite system consisting of Chinese cabbage, the fungus *A. brassicae*, and the mustard leaf beetle neither 1 nor 3 days of feeding resulted into any effect of the feeding damage on the fungus (Rostás & Hilker in press).

Not only the duration of herbivore feeding and the lag time between herbivore and fungal attack needs consideration, but also the sequence of herbivore and fungus occurrence may be important. Russo and co-workers (Russo et al. 1997) compared the herbivore's effect on the fungal pathogen by applying the herbivore either before or after the inoculation on the plant

(watermelon). This procedure also allows to reveal effects on later stages of the pathogen. Regardless of whether thrips or aphids were tested, the resistance effect on the pathogen was weakened when the herbivore was applied to the plant after inoculation compared to the resistance effect when the herbivore damaged the plant prior to inoculation. This result suggests that particularly early stages of the pathogen have been affected by herbivore feeding damage.

4.4 Specificity of herbivore-induced effects on fungal phytopathogens

Induced defensive responses of plants against herbivory may vary with the herbivore species attacking the plant (Stout & Bostock 1999 and references therein). This response specificity may be due on one hand to quantitative and qualitative differences in feeding when different plant tissues are damaged by e.g. phloem-sucking aphids and grazing lepidopteran larvae. On the other hand, also elicitors released by the herbivore during feeding are well-known to induce plant responses that clearly differ from purely mechanical damage (Turlings et al. 1993; Mattiacci et al. 1995; Hilker & Meiners in press). Nevertheless, so far there is no evidence that the specificity of a plant's response towards a herbivore is linked to specific effects on phytopathogenic fungi. Instead, plant damage caused by a single herbivore species may result in resistance against several phytopathogenic fungi. For example, defoliation of soybean by larvae of the soybean looper impaired two fungus species of soybean (Padgett et al. 1994). Feeding of the leaf beetle *G. viridula* upon *R. obtusifolius* negatively affected three different fungal species on dock plants (Hatcher & Paul 2000). However, while feeding damage by *G. viridula* could systemically reduce growth of a rust fungus on dock plant, mechanical damage caused only local effects on the rust (Hatcher et al. 1994b).

5. Induced plant physiological changes and their effects on herbivores and fungal phytopathogens

The two very different types of plant antagonists, phytopathogens and herbivores, are well-known to induce a great variety of local and systemic changes in plant primary and secondary biochemistry. In his excellent review, Hatcher (1995) considers comprehensively physiological changes of fungus-infected plants that might affect herbivorous insects and, vice versa, changes in herbivore-damaged plants that might have an impact on plant pathogens.

As already touched introductory, the two major signal transduction pathways triggered in response to pathogen and herbivore attack are the salicylate and jasmonate pathway accompanied by transduction systems with wound signals others than jasmonic and salicylic acid (Sticher et al. 1997; Stout & Bostock 1999; Bostock et al. 2001). For instance, ethylene produced in response to both, infection by pathogens and infestation by herbivorous insects appears to modulate to some extent the salicylate-induced expression of resistance against pathogens (Sticher et al. 1997) as well as the jasmonate-induced defensive response towards herbivores (Baldwin et al. 2001). If either one type of plant antagonist has induced wound signals and signal transduction in a plant, the compounds of the elicited pathways may not only affect the attacking type, but also the other one. Furthermore, signal transduction triggered by one type of attacker may interfere with signal cascades induced by the other (Preston et al. 1999, Stout et al. 1999, Thaler 1999a). Thus, the interference of signal transduction pathways induced by the plant pathogen and the herbivore may play a significant role on the effects of plant responses to either type of plant enemy (Paul et al. 2000).

Most of the plant changes studied so far have been detected in dual systems focusing on either plant-herbivore or plant-fungus interactions. In the following subchapter we highlight exemplarily several studies on tripartite plant-fungus-herbivore systems that attempted to link physiological changes of a plant caused by either herbivores or fungal pathogens with cross-effects on the antagonists. Nevertheless, all of these studies suffer from the fact that although plant changes may be associated with effects on the antagonist, proof of the causal relationship between induced responses and observed effects is almost lacking.

5.1 Fungus-induced plant physiological changes and their effects on herbivores

Changes in primary metabolites were found in rust-infected leaves of the dock plants *R. obtusifolius* and *R. crispus*. It is unknown whether the observed decrease in nitrogen content and the small rise in carbohydrates, resulting in an increased C/N ratio, is responsible for the detrimental effects of fungus-infected leaves on the performance of the leaf beetle *G. viridula*. Fungus-infected leaves of dock plants also show a significantly higher calcium oxalate concentration than healthy leaves. The elucidation of the impact of calcium oxalate

concentrations on the performance of *G. viridula* needs further studies (Hatcher et al. 1995b; Hatcher 1995).

The role of fungus-induced changes in primary and secondary metabolites in rust-infected willow on the performance of the willow leaf beetle *P. versicolora* is puzzling. Even though feeding on rust-infected willow leaves had detrimental effects on the performance of the willow leaf beetle *P. versicolora* (see 3.2.1), no changes in total carbohydrates and nitrogen could be made responsible for this observation. Likewise, only minor alterations in secondary metabolism were found. The salicin content in fungally infected leaves was slightly increased, whereas the concentration of another, potentially detrimental phenolglycoside, chlorogenic acid, remained unaffected (Simon & Hilker, unpublished data). Hence, other factors, yet unconsidered, must be held responsible for the observed effects.

Fungus-induced changes in primary metabolites in Chinese cabbage could not be linked with the negative effects of the plant's fungal infection on the performance of the mustard leaf beetle (see 3.2.1). Restricted infection with the causal agent of black spot disease *A. brassicae* did not induce significant changes in leaf water content, the ratio of carbon/nitrogen or in sucrose and total protein concentrations. However, changes in secondary metabolism were found in fungus-infected Chinese cabbage leaves, such as higher concentrations of indole glucosinolates and anthocyanins and an increased peroxidase activity (Rostás et al. submitted). Elevated levels of peroxidase activity are usually associated with systemic acquired resistance (SAR) which refers to the ability of a plant to impair the development of a phytopathogen after having previously been challenged with the same pathogen or a different one (e.g. Sticher et al. 1997). In the tripartite system consisting of Chinese cabbage, *A. brassicae*, and the mustard leaf beetle, no increase of peroxidase activity was found in leaves damaged by conspecific herbivores, whereas elevated glucosinolate and anthocyanin levels were also detected in herbivore-damaged leaves. However, in contrast to fungally infected plants, leaves damaged by conspecifics had no effect on larval development of *P. cochleariae*. Thus, the increased peroxidase activity of fungus-infected leaves might be a crucial factor to affect larval performance of *P. cochleariae* (Rostás et al. submitted).

In contrast, systemic induction of peroxidase activity in tomato by *Phytophthora infestans* did not affect the performance of *H. zea* larvae since feeding of larvae on fungus-infected

tomato leaves had no visible effect on larval performance. Neither did the induction of pathogenesis-related proteins by the fungus affect the herbivore (Stout et al. 1999). Locally and systemically increased peroxidase activities were also detected in fungus-infected cucumber leaves. However, cucumber leaf beetles removed even more leaf material from locally infected leaves, but not from systemic ones. Aphid reproduction was enhanced on locally infected leaves. Systemically infected cucumber leaves also showed enhanced concentrations of phenolics, but locally infected leaves did not (Moran 1998).

Fungus-induced phytoalexins may not only act as antifungal agents, but have also been linked with insect resistance (Hatcher 1995). For example, Mexican bean beetles avoided feeding upon soybean leaf tissue with elevated phytoalexin concentrations (Hart et al. 1983).

5.2 Herbivore-induced plant physiological changes and their effects on fungal phytopathogens

Many chemicals induced by insect feeding have antimicrobial activities and, thus, may act against phytopathogens (Krischik et al. 1991). The physiological changes induced by feeding damage may be influenced by the herbivore's feeding behaviour and by the release of oral secretions by the herbivore (Felton & Eichenseer 1999). For example, larval regurgitant of *Manduca sexta* that comes into contact with wounded tobacco leaves elicits an ethylene burst in the plant that downregulates the production of nicotine induced by the wound process *per se* (Kahl et al. 2000). Up to now, numerous studies are available that address the molecular and chemical base of changes in plant metabolism in response towards herbivory (see this issue and references therein).

In contrast, few studies to date have tried to link the effects of herbivore-induced plant responses on phytopathogenic fungi with physiological changes in the plant. Stout et al. (1998, 1999) could show that infestation of tomato plants with larvae of *Helicoverpa zea* induced polyphenol oxidase and proteinase inhibitor activity. Tomato leaves that had been fed upon by *H. zea* were more resistant against the causal agent of bacterial speck disease of tomato, *Pseudomonas syringae*. Whether feeding of the moth larvae also affects fungal disease of tomato has not been tested in this study. Chinese cabbage plants damaged by feeding mustard leaf beetles *P. cochleariae* did not affect the fungus *A. brassicae*, even

though damaged leaves showed increased levels of glucosinolates and anthocyanines and reduced sucrose content in damaged leaves (Rostás et al. submitted).

6. Conclusions and perspectives

Interactions between phytopathogenic fungi and herbivores on a common host plant are highly complex. The outcome may depend on several interrelated factors: i) the ability of the plant to induce resistance and/or to tolerate attacking fungi or herbivores, ii) the biology of the species involved, iii) abiotic stresses that have not been addressed here, but are well-known to affect plant - herbivore or plant - fungus interactions, and iv) the timing and the spatial scale of the interaction. Despite some advances made in this research field, our knowledge about the mechanisms that shape tripartite interactions is still too limited to be of any predictive value. Maybe the only cautious generalisation which can be made, is that in the majority of cases previous attack did not enhance the suitability of the plant for a successive attacker. Also, the few studies intending to link cross-effects of herbivore damage and plant fungal disease with physiological and molecular mechanisms do not yet allow for generalisations of the mechanisms. Both nutrient allocation due to plant attack and cross-interactions between induced plant defensive mechanisms may be important for the outcome of cross-effects between a given herbivore and pathogen species.

Future research should try to discern the direct impact of fungi or herbivory from induced plant-mediated effects. Ecological studies should couple with measuring chemical changes within the plant and studies must show that the observed physiological alterations are responsible for enhanced resistance or susceptibility. Genetically modified plants should be a very helpful tool in this respect. The temporal and spatial aspects of interactions between plants and their natural enemies need more attention as they may be decisive for the outcome of such interactions. Furthermore, costs and benefits of induced defensive responses have been studied only in cases where the plant defends against one type of antagonist (Agrawal 1998; Baldwin 1998; van Dam & Baldwin 1998, Thaler 1999b; Redman et al. 2001). In which way a plant is able to "economically" defend itself against co-occurring attacks by herbivores and pathogens, maybe by deploying chemicals with broad effects against arthropods and microbes alike, is a question that can only be speculated about, but not be answered yet. Finally, the impact of pathogens on plant volatiles, and thus on the indirect defence against

herbivores, remains virtually unexplored. Changes in the plant's volatile bouquet may result from negative cross-talk as higher concentrations of salicylic acid, due to pathogen infection, could interfere with the octadecanoid-dependent volatile synthesis (Hunter 2000). Therefore, herbivores may be influenced in their host finding behaviour (Schütz et al. 1997). On the other hand, parasitoids and predators, which rely on plant volatiles, may not find their herbivorous prey, rendering the indirect induced defence of the plant useless. Although studies will inevitably get more complex, the third trophic level should not be forgotten.

7. References

- Agrawal AA (1998) Induced responses to herbivory and increased plant performance. *Science* 279: 1201-1202.
- Agrawal AA, Tuzun S, Bent E (eds) (1999) *Induced Plant Defenses Against Pathogens and Herbivores*. APS Press, St. Paul.
- Ajlan AM, Potter DA (1991) Does immunization of cucumber against anthracnose by *Colletotrichum lagenarium* affect host suitability for arthropods? *Entomologia Experimentalis et Applicata* 58: 83-91.
- Ayres PG (1992) *Pests and Pathogens - Plant Responses to Foliar Attack*. Bios Scientific Publishers, Oxford.
- Baldwin IT (1998) Jasmonate-induced responses are costly but benefit plants under attack in native populations. *Proceedings of the National Academy of Sciences* 95: 8113-8118.
- Baldwin IT, Halitschke R, Kessler A, Schittko U (2001) Merging molecular and ecological approaches in plant-insect interactions. *Current Opinion in Plant Biology* 4: 351-358.
- Baldwin IT, Preston, CA (1999) The eco-physiological complexity of plant responses to insect herbivores. *Planta* 208: 137-145.
- Bi JL, Murphy JB, Felton GW (1997) Antinutritive and oxidative components as mechanisms of induced resistance in cotton to *Helicoverpa zea*. *Journal of Chemical Ecology* 23: 97-117.
- Bostock, RM (1999) Signal conflicts and synergies in induced resistance to multiple attackers.

Physiological and Molecular Plant Pathology 55: 99-109.

Bostock RM, Karban R, Thaler JS, Weymann PD, Gilchrist D (2001) Signal interactions in induced resistance to pathogens and insect herbivores. *European Journal of Plant Pathology* 107: 103-111.

Boyle C, Aust HJ (1997) Ontogenetically determined resistance (adult plant resistance). In: Hartleb Heitefuss R, Hoppe, HH (eds) *Resistance of Crop Plants Against Fungi*. Fischer Verlag, Jena.

Carruthers RI, Bergstrom GC, Haynes P.A. (1986) Accelerated development of the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae), induced by interactions with *Colletotrichum graminicola* (Melanconiales: Melanconiaceae), the causal fungus of maize anthracnose. *Annals of the Entomological Society of America* 79: 385-389.

De Nooij MP, Biere A, Linders EGA (1992) Interactions of pests and pathogens through host predisposition. In: Ayres, PG (ed) *Pests and Pathogens - Plant Responses to Foliar Attack*. Bios Scientific Publishers, Oxford, pp 143-160.

Dicke M (2000) Chemical ecology of host-plant selection by herbivorous arthropods: a multitrophic perspective. *Biochemical Systematics and Ecology* 28: 601-617.

Felton GW, Eichenseer H (1999) Herbivore saliva and its effects on plant defense against herbivores and pathogens. In: Agrawal AA, Tuzun S, Bent E (eds) *Induced Plant Defenses Against Pathogens and Herbivores*. APS Press, St. Paul, pp 19-36.

Felton GW, Korth KL (2000) Trade-offs between pathogen and herbivore resistance. *Current Opinion in Plant Biology* 3: 309-314.

Felton GW, Korth KL, Bi JL, Wesley SV, Huhman DV, Mathews MC et al (1999) Inverse relationship between systemic resistance of plants to microorganisms and to insect herbivory. *Current Biology* 9: 317-320.

Fidantsef AL, Stout MJ, Thaler JS, Duffey SS, Bostock RM (1999) Signal interactions in pathogen and insect attack: expression of lipoxygenase, proteinase inhibitor II, and pathogenesis-related protein P4 in the tomato, *Lycopersicon esculentum*. *Physiological and Molecular Plant Pathology* 54: 97-114.

Friedli J, Bacher S (2001) Mutualistic interaction between a weevil and a rust fungus, two parasites of the weed *Cirsium arvense*. *Oecologia*, <http://dx.doi.org/10.1007/s004420100763>

Hammerschmidt R (1999) Induced disease resistance: how do induced plants stop pathogens? *Physiological and Molecular Plant Pathology* 55: 77-85.

Hammerschmidt R, Nicholson, RL (1999) A survey of plant defense responses to pathogens. In: Agrawal, AA, Tuzun, S, Bent, E (eds) *Induced Defenses Against Pathogens and Herbivores*. APS Press, St. Paul, pp. 55-72.

Hammond AM, Hardy TN (1988) Quality of diseased plants as hosts for insects. In: Heinrichs EA (ed) *Plant Stress - Insect Interactions*. John Wiley & Sons, New York, pp 381-432.

Hart SV, Kogan M, Paxton JD (1983) Effect of soybean phytoalexins on the herbivorous insects Mexican bean beetle and soybean looper. *Journal of Chemical Ecology* 9: 657-672.

Hatcher PE (1995) Three-way interactions between plant pathogenic fungi, herbivorous insects and their host plants. *Biological Review* 70: 639-694.

Hatcher PE, Ayres PG (1997) Indirect interactions between insect herbivores and pathogenic fungi on leaves. In: Gange AC, Brown VK (eds) *Multitrophic Interactions in Terrestrial Systems*. Blackwell Science, Oxford, pp 133-149.

Hatcher PE, Ayres PG, Paul ND (1995a) The effect of natural and simulated insect herbivory, and leaf age, on the process of infection of *Rumex crispus* L. and *R. obtusifolius* L. by *Uromyces rumicis* (Schum.) Wint. *New Phytologist* 130: 239-249.

Hatcher PE, Ayres PG, Paul ND, Whittaker JB (1994a) The effect of an insect herbivore and a rust fungus individually, and combined in sequence, on the growth of two *Rumex* species. *New Phytologist* 128: 71-78.

Hatcher PE, Paul ND (2000) Beetle grazing reduces natural infection of *Rumex obtusifolius* by fungal pathogens. *New Phytologist* 146: 325-333.

Hatcher PE, Paul ND, Ayres PG, Whittaker JB (1994b) Interactions between *Rumex* spp., herbivores and a rust fungus: *Gastrophysa viridula* grazing reduces subsequent infection by *Uromyces rumicis*. *Functional Ecology* 8: 265-272.

Hatcher PE, Paul ND, Ayres PG, Whittaker JB (1995b) Interactions between *Rumex* spp., herbivore and a rust fungus: the effect of *Uromyces rumicis* infection on leaf nutritional quality. *Functional Ecology* 9: 97-105.

Hatcher PE, Paul ND, Ayres PG, Whittaker JB (1994c) The effect of a foliar disease (rust) on the development of *Gastrophysa viridula* (Coleoptera: Chrysomelidae). *Ecological Entomology* 19: 349-360.

Haukioja E (1990a) Induction of defenses in trees. *Annual Review of Entomology* 36: 25-42.

Haukioja E (1990b) Positive and negative feedbacks in insect-plant-interactions. In: Watt AD (ed) *Population Dynamics of Forest Insects*. Intercept, Andover, pp. 113-122.

Heil M (2001) The ecological concept of costs of induced systemic resistance (ISR). *European Journal of Plant Pathology* 107: 137-146.

Hilker M, Meiners T (in press) Induction of plant responses towards oviposition and feeding of herbivorous arthropods: a comparison. *Entomologia Experimentalis et Applicata*.

Hunter MD (2000) Mixed signals and cross-talk: interactions between plants, insects and plant pathogens. *Agricultural and Forest Entomology* 2: 155-1.

Kahl J, Siemens DH, Aerts RJ, Gäbler R, Kühnemann F, Preston CA, Baldwin IT (2000) Herbivore-induced ethylene suppresses a direct defense but not a putative indirect defense against an adapted herbivore. *Planta* 210: 336-342.

Karban R, Baldwin IT (1997) *Induced plant responses to herbivory*. Chicago University Press, Chicago.

Karban R, Kuc J (1999) Induced resistance against pathogens and herbivores: an overview. In: Agrawal AA, Tuzun S, Bent E (eds) *Induced plant defenses against pathogens and herbivores*. APS Press, St. Paul, pp 1-15.

Klepzig KD, Robison DJ, Smalley EB, Raffa KF (1997) Effects of feeding by two folivorous arthropods on susceptibility of hybrid poplars clones to a foliar pathogen. *The Great Lakes Entomologist* 30: 99-104.

Kluth S, Kruess A, Tschardt T (2001) Interactions between the rust fungus *Puccinia*

punctiformis and ectophagous and endophagous insects on creeping thistle. *Journal of Applied Ecology* 38: 548-556.

Kok LT, Abad RG, Baudoin ABAM (1996) Effects of *Puccinia carduorum* on musk thistle herbivores. *Biological Control* 6: 123-129.60.

Kombrink E, Somssich IE (1995) Defense responses of plant to pathogens. *Advances in Plant Pathology* 21: 1-33.

Kombrink E, Schmelzer, E (2001) The hypersensitive response and its role in local and systemic disease resistance. *European Journal of Plant Pathology* 107: 69-78.

Krischik VA (1991) Specific or generalized plant defense: reciprocal interactions between herbivores and pathogens. In: Barbosa P, Krischik, VA, Jones CG (eds) *Microbial Mediation of Plant-Herbivore Interactions*. Wiley, New York, pp. 309-340.

Lappalainen J, Helander ML, Palokangas P (1995) The performance of the autumnal moth is lower on trees infected by birch rust. *Mycological Research* 99: 994-996.

Lucas, JA (1998) *Plant Pathology and Plant Pathogens*. Blackwell Science, Oxford.

Maleck K, Dietrich RA (1999) Defense on multiple fronts: how do plants cope with diverse enemies? *Trends in Plant Science* 4: 215-219.

Massei G, Hartley SE (2000) Disarmed by domestication? Induced responses to browsing in wild and cultivated olive. *Oecologia* 122: 225-231.

Mattiacci L, Dicke M, Posthumus MA (1995) β -Glucosidase: an elicitor of herbivore induced plant odor that attract host-searching parasitic wasps. *Proceedings of the National Academy of Sciences USA* 92: 2036-2040.

Meiners T, Hilker M (1997) Host location in *Oomyzus gallerucae* (Hymenoptera: Eulophidae), an egg parasitoid of the elm leaf beetle *Xanthogalerucae luteola* (Coleoptera: Chrysomelidae). *Oecologia* 112: 87-93.

Meiners T, Hilker M (2000) Induction of plant synomones by oviposition of a phytophagous insect. *Journal of Chemical Ecology* 26: 221-232.

Mondy N, Corio-Costet M-F (2000) The response of the grape berry moth (*Lobesia botrana*)

to a dietary phytopathogenic fungus (*Botrytis cinerea*): the significance of fungus sterols. *Journal of Insect Physiology* 46: 1557-1564.

Moran P (1998) Plant-mediated interactions between insects and a fungal plant pathogen and the role of plant chemical responses to infection. *Oecologia* 115: 523-530.

Moran P, Schultz JC (1998) Ecological and chemical associations among late-season squash pests. *Environmental Entomology* 27: 39-44.

Padgett GB, Russin JS, Snow JP, Boethel DJ, Berggren GT (1994) Interactions among the soybean looper (Lepidoptera: Noctuidae), threecornered alfalfa hopper (Homoptera Membracidae), stem canker, and red crown rot in soybean. *Journal of Entomological Science* 29: 110-119.

Paul ND, Hatcher PE, Taylor JE (2000) Coping with multiple enemies: an integration of molecular and ecological perspectives. *Trends in Plant Science* 5: 220-225.

Preston CA, Lewandowski C, Enyedi AJ, Baldwin IT (1999) Tobacco mosaic virus inhibits wound-induced jasmonic acid-mediated responses within but not between plants. *Planta* 209: 87-95.

Redman, A. M., D. F. Cipollini, Jr. & J. C. Schultz, 2001. Fitness costs of jasmonic acid-induced defense in tomato, *Lycopersicon esculentum*. *Oecologia* 126: 380-385.

Rostàs M, Bennett R, Hilker M (submitted) Comparison of induced physiological responses in Chinese cabbage towards herbivory and fungal infection.

Rostàs M, Hilker M (in press) Asymmetric plant-mediated cross-effects between a herbivorous insect and a phytopathogenic fungus. *Agricultural and Forest Entomology*.

Rostàs M, Hilker M (submitted, a) Indirect interactions between a phytopathogenic fungus and an entomopathogenic fungus in support of the slow-growth, high-mortality hypothesis.

Rostàs M, Hilker M (submitted, b) Feeding damage by larvae of the mustard leaf beetle deters conspecific females from oviposition and feeding.

Russo VM, Russo BM, Peters M, Perkins-Veazie P, Cartwright B (1997) Interaction of *Colletotrichum orbiculare* with thrips and aphid feeding on watermelon seedlings. *Crop*

Protection 16: 581-584.

Schoonhoven LM, Jermy T, van Loon JJA (1998) *Insect-Plant Biology*. Chapman and Hall, London.

Schütz S, Weißbecker B, Klein A, Hummel HE (1997) Host plant selection of the Colorado potato beetle as influenced by damage induced volatiles of the potato plant. *Naturwissenschaften* 84: 212-217.

Siemens DH, Mitchell Olds T (1996) Glucosinolates and herbivory by specialists (Coleoptera: Chrysomelidae, Lepidoptera: Plutellidae): Consequences of concentration and induced resistance. *Environmental Entomology* 25: 1344-1353.

Staples RC, Hoch HC, Epstein L, Laccetti L, Hassouna S (1985) Recognition of host morphology by rust fungi: responses and mechanisms. *Canadian Journal of Plant Pathology* 7: 314-322.

Sticher L, Mauch-Mani B, Métraux JP (1997) Systemic acquired resistance. *Annual Review of Phytopathology* 35: 235-270.

Stout MJ, Bostock RM (1999) Specificity of induced responses to arthropods and pathogens. In: Agrawal, AA, Tuzun, S, Bent, E (eds) *Induced Defenses Against Pathogens and Herbivores*. APS Press, St. Paul, pp. 183-211.

Stout MJ, Fidantsef AL, Duffey SS, Bostock RM (1999) Signal interactions in pathogen and insect attack: systemic plant-mediated interactions between pathogens and herbivores of the tomato, *Lycopersicon esculentum*. *Physiological and Molecular Plant Pathology* 54: 115-130.

Stout MJ, Workman KV, Bostock RM, Duffey SS (1998) Specificity of induced resistance in the tomato, *Lycopersicon esculentum*. *Oecologia* 113: 74-81.

Thaler JS (1999a) Jasmonic acid mediated interactions between plants, herbivores, parasitoids, and pathogens: a review of field experiments in tomato. In: Agrawal, AA, Tuzun, S, Bent, E (eds) *Induced Defenses Against Pathogens and Herbivores*. APS Press, St. Paul, pp. 319-334.

Thaler JS (1999b) Induced resistance in agricultural crops: Effects of jasmonic acid on herbivory and yield in tomato plants. *Environmental Entomology* 28: 30-37.

Tinney GW, Hatcher PE, Ayres PG, Paul ND, Whittaker JB (1998) Inter- and intra- species differences in plants as hosts to *Tyria jacobea*. *Entomologia Experimentalis et Applicata* 88: 137-145.

Turlings TCJ, McCall PJ, Alborn HT, Tumlinson JH (1993) An elicitor in caterpillar oral secretions that induces corn seedlings to emit chemical signals attractive to parasitic wasps. *Journal of Chemical Ecology* 19: 412-425.

Van Dam N, Baldwin IT (1998) Costs of jasmonate-induced responses in plants competing for limited resources. *Ecology Letters* 1: 30-33.

Wagner S, Boyle C (1995) Changes in carbohydrate, protein and chlorophyll content, and enzyme activity during the switch from uredinio- to teliospore sporulation in the bean-rust fungus *Uromyces appendiculatus* (Pers.) Link. *Journal of Phytopathology* 143: 633-638.