# Summary

A very large number of insects and fungi are specialised on using plants as a source of nutrients. Therefore, it is not seldom that herbivorous insects and pathogenic fungi will attempt to exploit the same individual host plant. Direct but also indirect, plant-mediated interactions between both type of attackers may take place in such a case. Indirect interactions may be the result of alterations in plant metabolism caused by insect infestation or pathogen infection. Thus, previous attack by either type of antagonist may have a beneficial or detrimental impact on the other antagonist type.

The present study investigated such tripartite interactions between the host plant Chinese cabbage (*Brassica rapa* ssp. *pekinensis*) and the two crucifer specialists *Phaedon cochleariae* (Coleoptera: Chrysomelidae) and *Alternaria brassicae* (Fungi imperfecti: Deuteromycetes). Both antagonist species attack the leaves of their host. Interactions between the phytopathogenic fungus and the herbivore were investigated both on a local (both antagonists on the same leaf) and systemic scale (antagonists on different leaves).

Experiments in the greenhouse assessed the effects of fungal infection of *B. rapa* ssp. *pekinensis* on the development and host selection behaviour of the leaf beetle *P. cochleariae*. Larvae fed with symptom-free parts of Chinese cabbage leaves were significantly slowed down in their development compared with larvae that fed upon leaves of healthy control plants or symptom-free (systemic) leaves of an infected plant. Furthermore, larvae on fungus-infected leaves had lower larval and pupal weights. However, no significant differences in overall mortality and fecundity were observed between larvae on infected, healthy or systemic leaves. Thus, the phytopathogenic fungus was found to have a negative influence on the herbivore´s performance on a local but not on a systemic scale (Chapter 2).

Subsequent experiments assessed whether the observed local and detrimental effects of plant fungal infection on herbivore performance would result into enhanced susceptibility of the affected larvae towards the entomopathogenic fungus *Metarhizium anispliae*. Larvae fed with *A. brassicae*-infected leaves and larvae fed with healthy Chinese cabbage leaves for three days were dipped into a suspension of *M. anisopliae* conidia ($LD_{50}$). As a result, 50 % of all larvae that were fed healthy leaves managed to pupate, while none of the larvae that had fed upon *A. brassicae*-infected leaves pupated but died before reaching the pupal stage. Since

larvae feeding on fungus-infected plants were smaller than those feeding on healthy plants, a second experiment investigated the role of larval size for their susceptibility towards the entomopathogen. When feeding on healthy plants, large larvae were found to be less susceptible towards *M. anisopliae* than small individuals of the same age. It can be concluded that larval size, although not the only factor, was decisive for the higher mortality caused by the entomopathogen. Furthermore, it was demonstrated that fungal infection of a plant could result into plant responses that enhances the herbivore´s suitability towards an entomopathogen (Chapter 3).

Combining the results described in chapter 2 and 3, they may provide support for the `slow-growth, high-mortality’ hypothesis - an idea much discussed among students of insect-plant biology. According to this hypothesis, herbivores face a higher risk of being killed by predators, parasitoids or pathogens as a consequence of slow larval growth. The reason for this is that herbivores might remain in the vulnerable stages for an extended period of time. As shown by the experiments in chapter 2, *P. cochleariae* larvae feeding on fungus-infected Chinese cabbage are smaller and have a prolonged developmental time (slow growth). Experiments outlined in chapter 3 revealed that slow growing larvae, feeding on diseased plants, suffer higher mortality from exposure to an entomopathogen (high mortality) than fast developing larvae feeding on healthy plants. Thus, these experiments indicate for the first time that a phytopathogenic fungus may indirectly influence an entomopathogenic fungus (Chapter 3).

Besides having an effect on the performance of beetle larvae, infection of Chinese cabbage by *A. brassicae* also influenced the host selection behaviour of *P. cochleariae* on a local scale. Adult leaf beetles avoided feeding and oviposition on diseased leaves in dual choice tests, while larvae actually preferred such leaves compared to healthy ones. In the performance tests the beetles had no contact to the fungus, however, this was possible in the choice tests here. Thus, direct effects or plant-mediated effects could have been responsible for the herbivore’s choice. Systemic effects, however, were not observed (Chapter 2).

Although fungal infection had a detrimental impact on the herbivore, no measurable effects were found the other way round. Herbivory did not result into any significant changes in the sizes of necrotic lesions caused by the phytopathogenic fungus on Chinese cabbage leaves.

Lesion size was independent of the lag time between herbivory and inoculation as well as the intensity of the feeding damage inflicted by second-instar larvae. Equally, no spatial effects on fungal growth (local/systemic) could be observed. In summary, the results of this study show an asymmetric relationship between herbivore and fungus, since fungal infection of the host plant influenced the herbivore but not vice versa (Chapter 2).

These ecological studies were supplemented by physiological assays to elucidate the mechanisms of the plant's response to insect attack and pathogen infection. Concentrations of components of plant primary (water, C/N ratio, sucrose, total protein) and secondary metabolism (glucosinolates, anthocyanins, peroxidase) were assessed. Both herbivory and fungal infection resulted in lower concentrations of leaf sucrose and increases in indole glucosinolates and anthocyanins. A significant but very small decrease in water content was observed in herbivore-damaged leaves only, while peroxidase activity was exclusively induced in fungus infected leaves. No measurable treatment effects on C/N ratio and total protein content were found after herbivory or fungal infection. Thus, apart from other, yet unknown, factors the increase in peroxidase activity may be held responsible for the adverse effect of fungally infected leaves on leaf beetle performance. This conclusion may be drawn since healthy Chinese cabbage leaves and leaves damaged by conspecific larvae did not differ in their nutritional value with regard to larval development (see below). (Chapter 4)

In addition to the studies of tripartite interactions between Chinese cabbage, *A. brassicae*, and *P. cochleariae*, dual plant-herbivore relationships were investigated. The following questions were addressed: a) Does feeding by second-instar *P. cochleariae* induce resistance in Chinese cabbage leaves against conspecific adult females and larvae? b) Are there insect-derived factors, such as the larval defence secretion, larval faeces or regurgitant which may directly or indirectly (by induction of plant responses) influence the feeding and oviposition behaviour of gravid *P. cochleariae*? Herbivory by second-instar larvae had no local nor systemic impact on the performance of the mustard leaf beetle. However, in a dual choice assay herbivore damaged leaves were significantly avoided for feeding and oviposition by gravid females when compared with leaves from an undamaged plant. In contrast, no effect was observed on second-instars. Also, there was no difference in the preference between leaves adjacent to those fed upon and leaves from a healthy plant. (Epi)chrysomelidial, the major component of the larval defence secretion was found to adsorb in minor quantities onto the leaf surface when larvae were caged onto Chinese cabbage leaves. The compound is known to deter female *P. cochleariae* from feeding and oviposition when encountered in larger quantities. However, this study showed that the amount of (epi)chrysomelidial that was released by feeding larvae and adsorbed to the leaf was not sufficient to deter females from feeding or egg laying. Furthermore, leaves that had been mechanically wounded were equally well accepted as healthy leaves by female beetles. Likewise, females did not significantly differentiate between healthy leaves and mechanically damaged leaves treated with larval faeces or regurgitant. In conclusion, it may be assumed that the observed preference of healthy over feeding damaged leaves by gravid females was the result of physiological plant changes induced by larval feeding activity (Chapter 5).

Interactions between plants, pathogens and herbivores are highly complex, depending on a variety of interrelated factors such as i) the ability of the plant to mount defensive responses or to tolerate attack to a certain degree, ii) the specific biologies of the species involved, iii) abiotic stresses (not considered in this study) and iv) the timing and the spatial scale of the interactions. The outcome of the interactions may be beneficial, detrimental or neutral to either side. This study showed that in the case of *A. brassicae* and *P. cochleariae* co-occuring on Chinese cabbage, only the leaf beetle had to cope with adverse effects induced by fungal attack while the fungus was not affected by prior herbivory. The plant responds to fungal and herbivore attack by overlapping and differential chemical responses. From these plant responses, the enhanced peroxidase activity observed in fungus-infected leaves is likely to have contributed to the poorer performance of the beetle larvae feeding on those leaves. Up to now, few studies like the present exist that attempted to link ecological and physiological data and tried to discern direct from plant-mediated local and systemic effects within a defined time frame. Further studies taking into consideration all these points are needed in order to achieve a better understanding of complex interactions like those between plants, fungi and herbivores.