

Chapter 1

General Introduction and Thesis Outline

Green plants are able to synthesise organic molecules using solar energy. Thus, they represent the first trophic level on which all heterotrophs on succeeding levels depend on. As a consequence, plants are continually exposed to primary consumers, with herbivorous insects and pathogenic fungi being the most abundant among them. For example, 60 described pathogens and an equal number of insect species are known to attack rice, one of the most important crop plants for human consumption in the world (de Nooij et al. 1992 and references therein).

However, plants, although immobile, are not helpless against this threat. Indeed, most plants stay healthy most of the time as they have evolved with pathogens and insect pests for millions of years. They possess several lines of defence against potential attackers of any kind (Lucas 1998). These plant defences may be constitutive or inducible, depending on whether the defensive trait is a pre-existing feature or a response to attack (Karban & Baldwin 1997).

Constitutive defences may consist of morphological obstacles, such as thorns that may fend off large mammals or trichomes that can kill minute herbivores like aphids. The cell wall of each plant cell represents an effective barrier against pathogenic invaders, in particular if it is impregnated with chemicals such as suberin and lignin (Lucas 1998). Additionally, plants produce and store an enormous variety of secondary metabolites (e.g. phenols, tannins, saponins) rendering plant tissue unpalatable or even toxic to many pathogens and herbivores (Harborne 1995).

Once an attack has occurred, plants may increase the concentrations of existing defence compounds or induce *de novo* production of defensive structures and chemicals. Such induced defences may be locally restricted to the site of attack. But often the information about an attack is transported systemically throughout the plant, inducing defensive responses also in unattacked plant parts (Baldwin et al. 2001). These induced responses may be addressed either directly against the attacker ('direct defense') or towards the third trophic level ('indirect

defence') (Baldwin & Preston 1999). Numerous studies show that plants may address the third trophic level by attracting predators and parasitoids of the herbivores (Dicke 2000).

Direct responses of plants against phytopathogens are e.g. the "hypersensitive response" where plant cells rapidly die at the site of fungal attack to stop and trap growth of penetration hyphae. In addition, phytoalexins (low-molecular-weight antimicrobial compounds) or pathogenesis-relevant proteins (e.g. chitinase) may inhibit further mycelial growth on a local or systemic scale, respectively (Hammerschmidt 1999). Direct plant defences against herbivorous arthropods seem to share many common features with antimicrobial defences (Hatcher 1995). Hypersensitive responses may also be elicited by egg laying or gall inducing arthropods (Hilker & Meiners in press). Secondary metabolites as e.g. glucosinolates - characteristic for Brassicaceae - may be induced by fungi and insects, both directly at the site of attack and in distant plant parts (systemic response). Such an increase in secondary metabolite concentration can also be effective against both types of plant antagonists, the fungus and the herbivore (Bennett & Wallsgrove 1994).

Indirect plant defences may be elicited by feeding (Dicke 2000; Turlings et al. 2000) or ovipositing herbivores (Meiners & Hilker 2000; Hilker & Meiners in press). In response to herbivore attack, the plant may change its odour in a way that lures the natural enemies of the herbivore. This has been interpreted as the plant's "cry for help". Elliot et al. (2000) asked whether a plant under herbivore attack might also indirectly favour entomopathogens as microbial representatives of the third trophic level by negatively affecting the physiological state of the herbivore and thus, enhancing its suitability towards pathogens.

Until recently, plant-pathogen and plant-herbivore interactions have been studied separately, being the subject of either plant pathologists or entomologists. This has done no justice to the fact that plants are often simultaneously challenged by pathogens and insects. Also, studies in this field used to have a purely ecological or molecular approach. However, there has been considerable rethinking since Hatcher's excellent review (Hatcher 1995) about ecological interactions between plants, phytopathogenic fungi and herbivorous arthropods on the one hand and the realisation that signal transduction pathways involved in induced resistance against insects and micro-organisms share much in common, on the other hand (Bostock et al. 2001). Within the last few years there has been enormous interest in integrating the different aspects of our knowledge about induced plant defences against multiple enemies

since it has been recognised that plant defences are highly complex and poorly understood. Also, it was acknowledged that plant responses to biotic attack may involve the allocation of primary metabolites, enabling the plant to regrowth and tolerance of attack (reviews: Bostock 1999, Karban & Kuc 1999, Maleck & Dietrich, Paul et al. 2000).

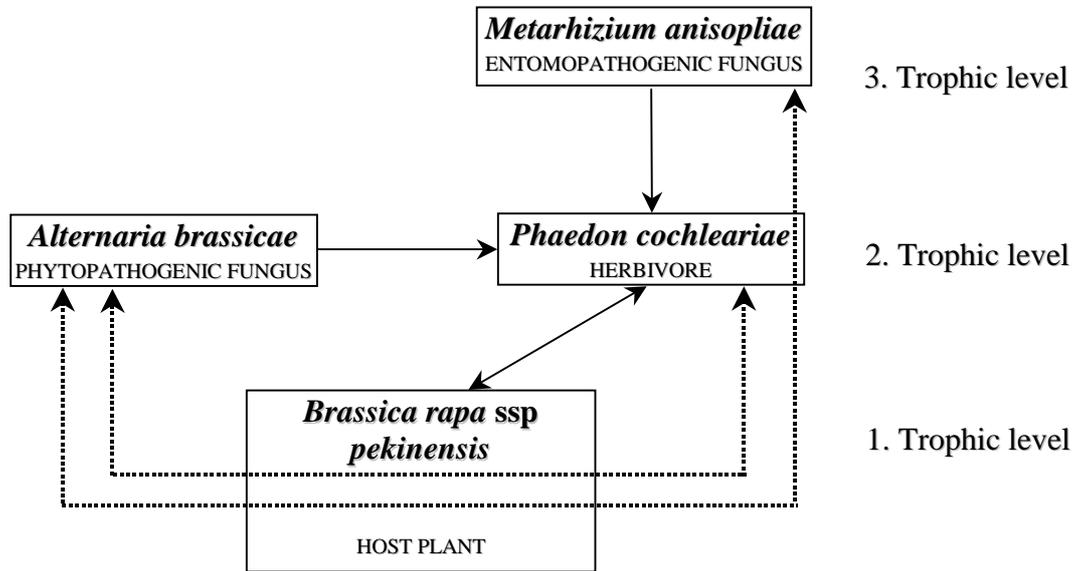


Figure 1 Representation of the direct (arrows with continuous line) and indirect (arrows with dotted line) interactions investigated in this thesis.

The thesis presented here focused on such plant responses against simultaneous or sequential challenge by herbivore and microbial attackers. Plant-mediated reciprocal interactions between the leaf beetle *Phaedon cochleariae* (Coleoptera: Chrysomelidae) and the phytopathogenic fungus *Alternaria brassicae* (Fungi imperfecti: Deuteromycetes) on one of their host plants Chinese cabbage (*Brassica rapa* spp. *pekinensis* cv. Kantonner) were investigated.

In chapter 2 the question was raised whether fungal infection of Chinese cabbage would induce local or systemic plant responses which may affect the host selection behaviour or development of *P. cochleariae* and vice versa, whether herbivory would result into any effects on the growth of the phytopathogen.

Chapter 3, going one step further, involved the third trophic level in this system. Experiments presented here assessed the indirect influence of plant pathogenic infection on the herbivore's enemy, the entomopathogenic fungus *Metarhizium anisopliae*. In particular,

the question was addressed whether *P. cochleariae* larvae feeding on fungus-infected plants suffer a higher suitability towards the entomopathogen when compared to larvae feeding on healthy plants.

These ecological studies were complemented in chapter 4 by physiological analyses on the mechanisms of the plant's response to fungal infection and herbivore infestation. Concentrations of various primary and secondary compounds were measured in healthy and attacked (fungus or herbivore) plants in order to gain some insight into the mechanistic causes that could have influenced the observed relationship between *A. brassicae* and *P. cochleariae* attacking Chinese cabbage.

In chapter 5 the relationship between *B. rapa* ssp. *pekinensis* and *P. cochleariae* was examined more closely. These investigations were necessary as they allowed a better interpretation of the chemical data obtained in chapter 4 and their relevance for the ecological observations described in chapter 2. The experiments presented here asked about the plant- and insect-derived factors which mediate the host selection of *P. cochleariae* and whether herbivory by this beetle would lead to induced resistance in Chinese cabbage against conspecific leaf beetles.

Finally, chapter 6 imbeds the knowledge gained in this thesis into the context of ecological studies on tripartite interactions between plants, phytopathogenic fungi and herbivorous insects. Special attention is paid in this review to the spatial and temporal aspects of such interactions.

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