**University of Natural Resources and Life Sciences, Vienna**

Department of Crop Sciences
Division of Plant Protection
Group of Arthropod Ecology and Behavior

# Mycorrhiza effects on the response of specialist and generalist predatory mites to spider mite-induced plant odors

## Master thesis

Master thesis for obtaining a Master's degree (Diplom-Ingenieurin) at the University of Natural Resources and Life Sciences, Vienna

**Submitted by**
Patricia Erler, Bakk.techn.

**Supervisor**
**Ao. Univ.-Prof. Dr. Peter Schausberger**

Vienna
November 2014

## ACKNOWLEDGEMENTS

First of all, I want to thank my entire family for their moral and financial support which has allowed me to receive the education I always desired. During my studies I was able to develop my own personality and grow according to my own wishes and ideas. Thank you for offering me this opportunity! Thank you for always supporting my ideas and wishes! Thank you for all your love!

Secondly, I offer enormous thanks to my supervisor Ao. Univ.-Prof. Dr. Peter Schausberger, who shared all his expertise and knowledge with me. His infinite support and patience during the whole Master thesis process enabled me to successfully complete my research. I could not have wished for a better supervision for my master thesis!

I would also like to thank Stefan Peneder and David Patiño-Ruiz from the laboratory of the Group of Arthropod Ecology and Behavior at the Division of Plant Protection for helping me with the setup of my experiments in the laboratory. Without their helpful advice the experiments would not have been successfully accomplished.

Thanks as well to my housemates Sylvia and Susi and all my other dear friends who always motivated and supported me during my studies and all the nice and funny times we spent together.

Last but not least, thanks to my boyfriend Günter for proofreading my thesis and all the motivating and encouraging words.

# TABLE OF CONTENTS

# 1. INTRODUCTION

Plants are growing in a sophisticated multitrophic environment and may have trophic relations to numerous other organisms both below- and aboveground. Such food webs usually consist of many interrelated interactions among species of different kingdoms and taxa and plants are playing a central role in these webs (Dicke and Vet 1999). With a few exceptions, the roots of nearly all plant species can form associations with mycorrhizal fungi, generally with benefits for both symbiotic partners (Hoeksema *et al.* 2010). Mycorrhizae may not only significantly change the host plant's chemistry, physiology and morphology by helping the plant to obtain nutrients that are otherwise inaccessible, especially phosphorus, from the soil, but also affect other soil microbes such as nitrogen-fixing bacteria. These interactions can be either direct by metabolic exchange or indirectly mediated by changes in the shared host plant (Smith and Read 2008). On the other hand, there are also interactions that negatively affect plant growth, both above- and belowground. Herbivory, for example, can decrease plant fitness by reducing the photosynthetic area needed to grow reproductive tissue, increase plant defense responses and even lead to the plants death (Smith and Read 2008). In the event of herbivore attack, plants commonly 'cry for help' by emitting herbivore-induced plant volatiles (HIPVs) to enhance recruitment of the herbivores' natural enemies (Dicke and van Loon 2000). The fact that above- and belowground plant-associated organisms interact was, until recently, largely disregarded in multitrophic studies and most scientists focused either on aboveground or on belowground organisms. However, to gradually improve our understanding of the ecology of plant growth and defense in multitrophic environments, we have to consider that belowground organisms have an impact on aboveground species and vice versa (van der Putten *et al.* 2011).

Recent scientific findings showed that arbuscular mycorrhizal fungi (AMF) may elicit changes in the blend of HIPVs released by bean plants in the event of attack by herbivorous spider mites (Schausberger *et al.* 2012). Additionally, AMF improve the quality of the spider mites as prey for their prime natural enemies, predatory mites (Hoffmann *et al.* 2011a). Predatory mites that are specialized on spider mite prey are able to perceive those mycorrhiza-induced changes in HIPVs, which ultimately benefits their own fitness (Hoffmann *et al.* 2011a; Schausberger *et al.* 2012).

Here, I pursued the hypothesis that only specialized spider mite predators are able to detect mycorrhiza-induced changes in HIPVs, because indicating higher prey quality, but not omnivorous diet generalists for which spider mites are only one of many possible food types.

## 1.1 Study organisms

### 1.1.1 Arbuscular mycorrhizal fungi (AMF)

The symbioses between arbuscular mycorrhizal fungi and autotrophic plants are generally, but not necessarily, regarded as mutualistic with benefits for both partners (Smith and Read 2008; Hoeksema *et al.* 2010). These symbioses are called arbuscular mycorrhizae (AM), which are ubiquitous phenomena in the natural environment of plant roots and are found in every terrestrial ecosystem on the planet (Koricheva *et al.* 2009). The fungi provide their host plants with mineral nutrients (especially phosphorus but, depending on the context, also nitrogen and others) through its hyphal network, receiving carbohydrates in return (Smith and Read 2008). Approximately 70 % of all land plants form symbioses with AMF (Hodge 2000), while the proportion of the world's flora associating with ectomycorrhizal fungi (EMF) amounts to 3 % (Smith and Read 2008). Currently, about 150 species of AMF are known, most of which are associating with a wide range of plants, whereas some plant species only associate with particular fungal symbionts (Hodge 2000).

The AM symbiosis consists of three important components: the root itself, the fungal structures within and between the cells of the root and an extraradical mycelium in the soil. Mycorrhizal colonization of roots can arise from spores, infected root fragments and hyphae (Smith and Read 2008). Hyphal contact with the root is followed by adhesion of the hypha to the root surface and with the formation of swollen appressoria after 2 to 3 days, followed by root penetration and formation of arbuscules after another 2 days (Rosewarne *et al.* 1997). Initially, hyphae grow intercellularly (Garriock *et al.* 1989), with arbuscules and vesicles forming after 5 to 10 days (Brundrett *et al.* 1985). Inside the plant cell, the forming main trunk of the arbuscule is invaginated in an apoplastic compartment outside the plant cell cytoplasm, with the result that the plasma membrane of the plant cell is not penetrated (Smith and Read 2008). Consequently, the AMF colonizes two different apoplastic compartments in the root, the intercellular spaces between cortical cells and a more specialized intracellular apoplast surrounding the coils (Smith and Read 2008). The transfer

of nutrients between the fungus and the plant occurs across symbiotic interfaces in these apoplastic compartments (Smith and Read 2008). Hyphae are also growing from the root into the soil and have two functions: to obtain nutrients from the soil and to establish secondary infections in other roots of the same or different plants (Rosewarne *et al.* 1997).

The AM-enhanced nutrient uptake depends on three essential processes: uptake of nutrients by the fungal mycelium in the soil, transportation within the hyphae to the arbuscules within the roots and transfer to the plant cells across the complex interface between the symbionts (Smith and Read 2008). The fungal mycelium enlarges the soil volume which can be naturally depleted for nutrients from the plants roots and penetrates smaller soil pores, resulting in context-dependent improved phosphorous (P), nitrogen (N), zinc (Zn) and/or copper (Cu) supply for the plant. Also the concentrations of potassium (K) in AMF plants are occasionally higher than those in non-infested plants, but depend on various factors as for instance the availability of N and P and the mobility of K-ions in the soil (Smith and Read 2008). A review published by Read and Perez-Moreno (2003) reported, that AMF might even be involved in direct attack upon nutrient containing organic polymers and may also be able to compete effectively with soil-inhabiting microorganisms for recently mineralized nutrients.

Mycorrhizal plants generally benefit from an improved nutrient uptake, which in turn may enhance plant growth, size, vigor etc. (Smith and Read 2008), but these benefits on plant performance greatly depend on plant and fungal genotypes and soil abiotic and biotic conditions (Hoeksema *et al.* 2010).

In return for providing nutrients to the plant, AMF are entirely dependent on an organic carbon (C) supply from their photosynthetic partner. Between 4 and 20 % of the plant's net photosynthate is transferred to the fungus and used in production of both vegetative and reproductive structures (Smith and Read 2008).

### 1.1.2 The arbuscular mycorrhizal fungus *Glomus mosseae*

*Glomus mosseae* (Nicol. & Gerd.), the fungus applied in this study, is a worldwide distributed, ubiquitous arbuscular mycorrhizal fungus able to form symbioses with a wide range of vascular plants (Smith and Read 2008). Several studies examined the effects of *G. mosseae* on its host plants (e.g. Ortas and Akpinar 2006). Regarding the host plant species used in my study, common bean *Phaseolus vulgaris* L., Isobe and Tsuboki (1999) discovered that the

symbiosis with *G. mosseae* exerted positive effects on the growth and grain yield of the bean plant. Hoffmann *et al.* (2009) examined the influence of *G. mosseae* on plant growth of *P. vulgaris* and the effects of an altered nutrient content on the two-spotted spider mite *Tetranychus urticae* Koch, a polyphagous herbivore. Hoffmann *et al.* (2009) recently showed that *T. urticae* benefits from increased P and K content levels in the leaves of its host plants and preferentially feeds and oviposits on mycorrhizal plants. Furthermore, AMF symbiosis shortens their egg developmental time and increases the oviposition rate and female offspring proportion. Patiño-Ruiz and Schausberger (2014) recently found that adaptive learning enables *T. urticae* to perceive mycorrhiza-induced changes in host plant volatiles leading the herbivores to a more favorable food source. The enhanced life history performance of *T. urticae* on mycorrhizal plants seems to be detrimental for both partners of the mycorrhizal symbiosis, the host plant and the AMF (Hoffmann *et al.* 2011b). However, Hoffmann *et al.* (2011a; 2011b) showed that AM may also affect indirect plant defense mechanisms via enhancing the performance of the prime natural enemies of the herbivores, predatory mites, and thereby render the bean plant-fungus symbiosis net positive for both partners, despite enhanced spider mite proliferation.

In my study system, spider mite feeding negatively affects root colonization by AMF (Hoffmann *et al.* 2011b) but the effects of herbivory on AMF are not always negative, also depending on whether herbivory occurs above- or belowground (Wamberg *et al.* 2003). For example, root herbivory by *Tipula paludosa* larvae on *Agrostis capillaris* changed the quantity and composition of root exudates, thus leading to increased colonization by *G. mosseae* and *G. intraradices* (Currie *et al.* 2006).

### 1.1.3 Linking above- and belowground interactions

Research during the last two decades revealed that plants are often simultaneously influenced by both above- and belowground organisms. However, most studies focused exclusively on aboveground tritrophic interactions, possibly also because interactions in the soil are not that easy to monitor, sample and manipulate experimentally. For a better understanding of plant defense mechanisms, above- and belowground plant-associated organisms need to be linked and the influence of belowground organisms such as root-feeding herbivores, soil pathogens or mycorrhizal fungi on aboveground organisms and the ecology of the plant as a whole has to be considered (reviewed by van der Putten *et al.* 2001).

Both above- and belowground organisms have an impact on trophic cascades which may be characterized by "top-down" and "bottom-up" forces. Top-down phenomena are, for example, caused by predators influencing species in a lower trophic level in the food chain. In contrast, interactions between e.g. primary producers and their root symbionts cascading up in trophic webs are typical bottom-up phenomena. Because all members of the web are directly or indirectly connected to each other, species at any trophic level vary in importance and flows can be upward and downward. This permits species to dominate the trophic system through feedback loops and pervasively influence all other species in the web (Hunter and Price 1992).

For my study system, Hoffmann *et al.* (2011a) discovered a bottom-up trophic cascade caused by changes in bean plants *P. vulgaris* triggered by the mycorrhizal fungus *G. mosseae*. The fungus induced plant-mediated changes in the prey quality of an aboveground herbivore, the spider mite *T. urticae*, which in turn resulted in increased fitness of the predatory mite *Phytoseiulus persimilis* Athias-Henriot feeding on the spider mites. The increased fitness of the predator cascaded down and compensated the negative effects of the mycorrhiza-enhanced life history performance of *T. urticae* (Hoffmann *et al.* 2009) to the fitness benefit of the host plant. Follow-up research (Hoffmann *et al.* 2011b) revealed a positive feedback loop on the AMF caused by the enhanced predator performance. Due to *P. persimilis* reducing the numbers of *T. urticae*, the roots supply with carbon was enhanced, thus leading to a higher root colonization level by *G. mosseae*. To my knowledge, the study by Hoffmann *et al.* (2011b) was the first experimental proof for a mutually beneficial interaction between a belowground AMF and an aboveground third trophic level enemy.

### 1.1.4 Plant defense and herbivore-induced plant volatiles

In 1980, Price *et al.* published a seminal review about the role of natural enemies of plant herbivores in plant defense. These authors highlighted the relevance of natural enemies as mutualists of plants and the importance of integrating third trophic level interactions in plant defense mechanisms.

Plant defense strategies are highly diverse and can be divided into direct and indirect defenses. Direct plant defense mechanisms are for example plant morphological structures such as trichomes and thorns or chemical compounds such as toxins and digestibility reducers, which negatively affect the performance of herbivores or pathogens. Indirect

defense strategies target the natural enemies of the pathogens or herbivores, for example by emitting attractive volatiles or enhancing the performance and effectiveness of the carnivores by providing food supplements or shelter (e.g. domatia). Induced indirect plant defense mechanisms include volatiles or food supplements that are not constitutively present but only produced in the event of plant attack by herbivores or pathogens. Inducible expression of defense minimizes the metabolic costs of the production of defense chemicals (reviewed by van der Putten *et al.* 2001, Bezemer and van Dam 2005).

Upon attack, plants are able to produce a diversity of secondary metabolites like for instance herbivore-induced plant volatiles (HIPVs). HIPVs are produced in response to herbivore attack or egg deposition to enhanced recruitment of the herbivores' natural enemies. These secondary plant metabolites may consist of hundreds of single compounds and mainly comprise terpenoids, fatty acid derivatives, phenyl propanoids and benzenoids. They are either produced *de novo* by attacked plants or, if also produced by undamaged plants, with quantitatively changed synthesis in case of infestation (Dicke and van Loon 2000). Furthermore, the induced release of the volatiles is commonly systemic and is not restricted to the site of damage (Dicke *et al.* 1990). Also mechanically damaged plants may change their volatiles, but in a different quantity and quality compared to volatiles induced by herbivory (Dicke and Vet 1999). Herbivore-induced and constitutively emitted blends of plant volatiles do not only differ in the quantity of volatiles released per unit of plant biomass but also in the composition of the volatile mixtures (Dicke and van Loon 2000). Some plants produce qualitatively similar chemical mixtures irrespective of the herbivore that feeds on them, therefore the specificity in plant response often lies in the quantity of blend components (Dicke and Vet 1999).

The released HIPVs can be specific for a given plant species attacked by a given herbivore species, but in many cases such plant volatiles also affect other plants, herbivore competitors or predators of the carnivores. This implies that the food web is overlaid with a more complex information web, because information transmission may occur irrespective of trophic relationships (Dicke and Vet 1999).

Up to now, indirect plant defense through the attraction of third trophic level predators was found in more than 49 plant species and at least 25 different predator species (Mumm and Dicke 2010). Research on HIPVs started in the 1980s, when Sabelis and van de Baan (1983) observed that the specialist predatory mites *P. persimilis* and *Metaseiulus occidentalis*

reacted positively to the odor stream emitted by leaves of lima bean plants *Phaseolus lunatus* infested with *T. urticae*. The generalist predatory mites *Amblyseius andersoni* (Chant) (= *A. potentillae*) and *Euseius finlandicus* (Oudemans) (=*Amblyseius finlandicus*) preferred the odor of apple leaves infested by the European Red Mite (*Panonychus ulmi*) to the odor of clean leaves. Hence, phytoseiid predators preying on or having a preference for a particular tetranychid species reacted to the odor of leaves infested by their preferred species, in order to maximize their reproductive success. Further research by Dicke *et al.* (1990) on similar tritrophic systems consisting of *P. lunatus*, *T. urticae* and the two carnivores *P. persimilis* and *A. andersoni* revealed that *P. persimilis* was especially attracted by the four herbivore-induced volatile compounds linalool, methyl salicylate, (*E*)-β-ocimene, and (*E*)-4,8-dimethyl-1,3,7-nonatriene, whereas the green leaf volatiles (*Z*)-3-Hexen-1-ol and (*Z*)-3-hexenyl acetate, which are released after herbivory as well as physical damage (Reddy *et al.* 2002), are not appealing for *P. persimilis*. To the contrary, *A. andersoni* only responded to linalool and methyl salicylate when reared on a carotenoid-deficient diet consisting of *Vicia faba* pollen (Dicke *et al.* 1990).

Koveos *et al.* (1995) found that *A. andersoni* responded positively to odors emanating from *P. vulgaris* infested with *T. urticae*, providing additional behavioral evidence for the plant being the producer of the carnivore attractants.

Shimoda *et al.* (2005) showed that also the generalist predatory mite *Neoseiulus californicus* (McGregor) may be attracted by the odor from leaves of *P. lunatus* infested with the spider mite *T. urticae*. The finding that significantly more predators preferred spider mite damaged leaves after removing *T. urticae* to artificially damaged leaves suggested that *N. californicus* is capable of perceiving a variety of plant-derived volatile infochemicals induced by their prey. Similar to *P. persimilis*, also *N. californicus* was attracted by both linalool and methyl salicylate (Shimoda *et al.* 2005).

As described, mutualistic interactions between plants and predatory arthropods are widespread and strongly suggest co-evolution (Dicke and Vet 1999). Volatiles that are attractive to carnivorous arthropods may be induced by herbivores from six different insect and mite orders: Lepidoptera, Diptera, Thysanoptera, Coleoptera, Hemiptera and Acari (Mumm and Dicke 2010). The tritrophic system worked with in my study, consisting of bean plants such as *P. lunatus* or *P. vulgaris*, *T. urticae* and predatory mites such as *P. persimilis* or

*N. californicus* is one of the best known and most often applied model system in research on HIPVs (e.g. Sabelis and van de Baan 1983; Dicke *et al.* 1990; Shimoda *et al.* 2005).

### 1.1.5 The effect of arbuscular mycorrhiza on herbivore-induced plant volatiles

Belowground organisms, such as AMF, can influence defense responses aboveground and, vice versa, aboveground organisms such as herbivores can influence belowground defenses (Bezemer and van Dam 2005). Studies on the impact of AMF on the metabolization of HIPVs are extremely scarce (Fontana *et al.* 2009; Leitner *et al.* 2010) and only Guerreri *et al.* (2004) and Schausberger *et al.* (2012) additionally focused on the associated attraction of AMF influenced plant volatiles to aboveground natural enemies of herbivores. The AMF *Glomus intraradices* induces quantitative and qualitative changes in the volatile compounds emitted by *Plantago lanceolata* (Fontana *et al.* 2009) and *Medicago truncatula* (Leitner *et al.* 2010) in case of herbivory by caterpillars of *Spodoptera* spp. Guerreri *et al.* (2004) linked the mycorrhizal symbiosis of *Glomus mosseae* and tomato plants with the increased attraction of the parasitic wasp *Aphidius ervi* to changes in plant-emitted volatiles. However, in the study by Guerreri *et al.* (2004) the parasitoids were more strongly attracted by mycorrhizal plants, irrespective of the presence of their hosts, the aphid *Macrosiphum euphorbiae*, and aphid feeding on the plant, respectively.

Schausberger *et al.* (2012) used the abovementioned well-known tritrophic system of the bean plant *P. vulgaris*, the spider mite *T. urticae* and the predator *P. persimilis* to investigate and link mycorrhiza-induced changes in HIPV emission and attraction of natural enemies. Their analysis using proton-transfer mass spectrometry revealed remarkable mycorrhiza-induced changes in the HIPV composition. Two compounds metabolized *de novo* in case of spider mite attack, β-ocimene and β-caryophyllene, were increasingly emitted by mycorrhizal plants whereas emission of the constitutively present methyl salicylate was increased by infestation with *T. urticae* but decreased in presence of mycorrhiza. However, no single compound was metabolized *de novo* following mycorrhization. The fact that the HIPVs of spider mite infested mycorrhizal plants were more attractive to *P. persimilis* than the HIPVs of spider mite-infested non-mycorrhizal plants allowed the conclusion that the predatory mite *P. persimilis* can discriminate mycorrhizal from non-mycorrhizal plants (Schausberger *et al.* 2012) and is more strongly attracted to mycorrhizal plants harboring more nutritious prey, and thus ultimately enhancing predator fitness (Hoffmann *et al.* 2011a).

### 1.1.6 The spider mite *Tetranychus urticae*

The two-spotted spider mite *Tetranychus urticae* belongs to the family of the true spider mites, the Tetranychidae. It is a worldwide distributed, highly polyphagous crop pest, causing serious plant damage, yield loss and even death of the plant (Helle and Sabelis 1985a). *T. urticae* may feed on the leaves of more than 800 plant species, including vegetables, fruit trees, maize and a wide range of ornamentals (van Leeuwen *et al.* 2010). The spider mites penetrate the leaf tissue with their stylets and suck out the parenchyma cell contents (Tomczyk and Kropczynska 1985). Spider mite feeding severely impairs the health of their host plants, because of the loss of mineral elements and loss of water by increased transpiration leading to water stress. However, most damage is caused by the loss of chlorophyll resulting in a reduced photosynthetic rate (Tomczyk and Kropczynska 1985). When searching for suitable host plants, *T. urticae* often exploits plant volatiles emitted by both herbivore-uninfested and infested plants (Dicke and van Loon 2000). The complex web produced by these spider mites protects them from many predator species and from desiccation under low relative humidity (Saito 1985).

Although biological control of *T. urticae* has proven successful in many crops, the use of insecticides and acaricides is still very common. The high fecundity (up to 10 eggs/female/day at 25 °C) and relatively short life cycle of *T. urticae* facilitate quick development of resistance to many chemicals used in food crops (van Leeuwen *et al.* 2010).

Table 1: Taxonomic classification of *T. urticae* (after Hoy 2011)

| **Taxonomic level** | **Name** |
|---|---|
| Kingdom | Animalia |
| Phylum | Arthropoda |
| Subphylum | Chelicerata |
| Class | Arachnida |
| Subclass | Acari |
| Order | Acariformes |
| Suborder | Actinedida |
| Family | Tetranychidae |
| Genus | *Tetranychus* |
| Species | *Tetranychus urticae* C. L. Koch 1836 |

### 1.1.7 The predatory mites

Many predatory mites of the family Phytoseiidae, including the predatory mite species used in my study, *P. persimilis*, *N. californicus*, *A. andersoni* and *E. finlandicus*, are very successful agents of biological control against the two-spotted spider mite *T. urticae* and the European Red Mite *Panonychus ulmi*, respectively. Approximately 2000 species belong to the family of Phytoseiidae, many of which are considered to be valued natural enemies of herbivorous mites and insects in diverse greenhouse and outdoor crops (e.g. Helle and Sabelis 1985b; McMurtry and Croft 1997). The Phytoseiidae represent the best and most comprehensively studied group of predatory mites used in biological control; their members may occur in all different kinds of natural and agricultural ecosystems. Depending on their diet specialization, they usually prey on other mites and small insects, but the generalists may also feed on pollen, honeydew, fungi or even suck plant liquids (Helle and Sabelis 1985b; McMurtry and Croft 1997).

Table 2: Taxonomic classification of *Phytoseiidae* (after McMurtry and Croft 1997)

| **Taxonomic level** | **Name** |
|---|---|
| Kingdom | Animalia |
| Phylum | Arthropoda |
| Subphylum | Chelicerata |
| Class | Arachnida |
| Subclass | Acari |
| Superorder | Parasitiformes |
| Order | Mesostigmata |
| Family | Phytoseiidae |

McMurtry and Croft (1997) proposed four major types of phytoseiid mites, with respect to their role as natural enemies of spider mites and differences in food utilization, respectively.

Fig. 1: *Phytoseiulus persimilis* female (right) hunting for *Tetranychus urticae* (left; © Krister Hall).

*Phytoseiulus persimilis* is categorized as a type I predator. It is specialized to feed on *Tetranychus* species and is well able to cope with the dense, irregular webs produced by these spider mites (McMurtry and Croft 1997). Morphological adaptations, e.g. long dorsal setae (Sabelis and Bakker 1992), and behavioral adaptations such as cutting the threads with the pedipalps and chelicerae (Shimoda *et al.* 2009), enables specialist predatory mites, such as *P. persimilis*, to move inside dense webs without getting entangled in the sticky silken strands. Schmidt (1976) found, that *P. persimilis* is more strongly attracted by the webs of *T. urticae* and associated chemical cues than by their eggs and exuviae and thus uses the silken threads for prey location. Furthermore, *P. persimilis* takes advantage from the dense webbings of their prey. It oviposits as well as resides inside the webs, which protects themselves and their progeny from intraguild predators like the western flower thrips, *Frankliniella occidentalis* (Roda *et al.* 2000). For its well-developed adaption on spider mites, *P. persimilis* became one of the most successful biological control agents on various crops such as beans, peaches and roses and is being used in many parts of the world (Gerson *et al*. 2003).

*Neoseiulus californicus* is also able, but less so than *P. persimilis*, to cope with the dense webbing of *T. urticae*. Accordingly, this species is categorized as a type II predator (McMurtry and Croft 1997). In addition to *T. urticae*, *N. californicus* also feeds on other spider mites, e.g. *Panonychus citri*, small insects such as thrips and on plant-derived substances such as pollen. Despite lesser specialization, *N. californicus* may be used, on

certain crops and under certain circumstances, to control spider mite pests (Gerson *et al.* 2003).

Type III and IV phytoseiids, such as *Amblyseius andersoni* (McMurtry *et al.* 2013) and *Euseius finlandicus* (McMurtry and Croft 1997), are described as broad diet generalists, but some species are still capable of controlling pestiferous spider mites (Gerson *et al.* 2003). Type III and IV predators do not aggregate in spider mite prey colonies as extensively as Type I and II predators do (Zhang *et al.* 1992). Due to their short mid-dorsal setae, they can get easily entangled in dense spider mite webbing and may consequently die of starvation (Sabelis and Bakker 1992).

McMurtry *et al.* (2013) categorized *A. andersoni* as a subtype III-b generalist predator living on glabrous leaves, feeding on different kinds of spider mites, pollen and fungi (McMurtry and Croft 1997; Pozzebon and Duso 2008).

*Euseius finlandicus* (Oudemans) is categorized as a type IV phytoseiid, meaning that it is a pollen-feeding generalist predator (McMurtry and Croft 1997). It has a high reproductive capacity when feeding on pollen, but can also live on eriophyoid and tetranychid species as well as on fungi (McMurtry and Croft 1997; Schausberger 1998; Pozzebon and Duso 2008). In contrast to many other *Euseius* species living in semi-tropical or tropical areas, *E. finlandicus* mostly occurs in temperate zones on deciduous shrubs and trees (Croft *et al.* 1998).

### 1.1.8 Study rationale and hypothesis

Plants are living in a complex multitrophic environment. As interactions between above- and belowground plant-associated organisms cannot be examined that easily, they have not yet been well studied and are still poorly understood (van der Putten *et al.* 2011). Commonly, *G. mosseae*-colonized plants benefit from an improved nutrient uptake (Isobe and Tsuboki 1999; Hoffmann *et al.* 2009). As a consequence, the life history performance of the herbivorous spider mite *T. urticae* is enhanced when feeding on mycorrhizal *P. vulgaris* plants, as compared to non-mycorrhizal plants (Hoffmann *et al.* 2009). The negative effects of increased spider mite performance on plant fitness may be outweighed by enhanced recruitment of the natural enemies of the spider mites, such as *P. persimilis* (Hoffmann *et al.* 2011a), through mycorrhiza-induced changes in the chemical composition of HIPVs emitted by the plant (Schausberger *et al.* 2012). In return, *P. persimilis* benefits from more nutritious

prey living on mycorrhizal plants, resulting in increased predator fitness (Hoffmann *et al.* 2011a). The fact that reduced numbers of spider mites increase fungal root colonization levels provides evidence of a mutually advantageous interaction of the AMF and the specialized predatory mite *P. persimilis* (Hoffmann *et al.* 2011b).

It is known that also the generalist *N. californicus* prefers spider mite-infested bean leaves to non-infested leaves and is attracted by the volatile infochemicals linalool and methyl salicylate originating from attacked bean plants (Shimoda *et al*. 2005).

Koveos *et al.* (1995) and Sabelis and van de Baan (1983) provided the behavioral proof that also the generalist *A. andersoni* is able to discriminate spider mite-infested bean and apple leaves from non-infested leaves.

Similarly, the generalist *E. finlandicus* has been reported to use plant volatiles for long distance detection of its prey mites. The volatiles emitted by apple leaves infested by the spider mite *Panonychus ulmi* (Sabelis and van de Baan 1983) and leaves of *Castanea* species infested by *Oligonychus ununguis* (Sun *et al.* 2002) allowed *E. finlandicus* to target these prey species.

However, while also the generalists *N. californicus*, *A. andersoni* and *E. finlandicus* are principally able to orient themselves on HIPVs, it is unknown whether they are, similar to *P. persimilis*, also able to discriminate HIPVs emitted by *T. urticae*-infested mycorrhizal plants from *T. urticae*-infested non-mycorrhizal plants. Considering prey-predator co-evolution and given the high specialization of *P. persimilis* on *T. urticae*, which contrasts the more generalized feeding habits of the other three predators, *N. califonicus*, *A. andersoni* and *E. finlandicus*, I hypothesized that only *P. persimilis* but not the generalist predators have been selected to perceive and recognize the subtle mycorrhiza-induced changes in HIPVs providing information of *T. urticae's* quality as prey.

In order to test my hypothesis, I performed experiments, using a Y-tube olfactometer, assessing the discrimination ability and preference of *P. persimilis*, *N. californicus*, *A. andersoni* and *E. finlandicus* given a choice between odors from *T. urticae*-infested mycorrhizal (+M) and non-mycorrhizal (-M) bean plants.

# 2. MATERIALS AND METHODS

For the experiments, four different species of predatory mites were reared, mycorrhizal and non-mycorrhizal bean plants were grown and the percentage of root length colonized by the mycorrhizal fungus (RLC) was estimated. All adult female predatory mites used in the experiments were pre-experimentally starved in acrylic cages and then given a choice between a mycorrhizal and non-mycorrhizal odor source in a Y-shaped olfactometer.

## 2.1 Rearing of predatory mites

The laboratory-reared population of *Phytoseiulus persimilis* originally derived from a population in Valencia/Spain, those of *Neoseiulus californicus* and *Amblyseius andersoni* came from Sicily/Italy and *Euseius finlandicus* was collected from horse-chestnut trees in Türkenschanzpark, Vienna/Austria.

Specimens of the four different species of predatory mites used in the experiments, *P. persimilis*, *N. californicus*, *A. andersoni* and *E. finlandicus,* were reared separately on detached leaves of non-mycorrhizal common bean plants *Phaseolus vulgaris.* Single primary bean leaves were placed upside down on moist filter paper covering a water-saturated foam cube located in a 20 x 20 cm plastic tray half filled with water. The leaf margins were covered with moist filter paper to prevent the mites from escaping (Figure 2). Leaves used to rear *E. finlandicus* were additionally provided with small pieces of a transparent plastic sheet (1 to 2 $cm^2$) folded in the shape of a tent with tufts of cotton threads underneath, serving as egg-laying sites and shelter (Kostiainen and Hoy 1994). Rearing units of *E. finlandicus* were stored at 25 ± 1 °C, 45 ± 5% rH and 16:8 h (light:dark period) L:D, those of the other three predator species were stored at 25 ± 1 °C, 60 ± 5 % rH and 16:8 h L:D.

*P. persimilis*, *N. californicus* and *A. andersoni* were fed every two days with two-spotted spider mites, reared on whole non-mycorrhizal bean plants under standardized environmental conditions (25 ± 5 °C, 60-80 % rH, 16:8 h L:D), by brushing the spider mites from infested bean leaves onto the rearing units. *E. finlandicus* was fed birch pollen (Schausberger 1992) by dusting pollen onto the leaves once a week. To avoid any effects induced by different pre-experimental diets and to familiarize the predatory mites to *T. urticae* prey, adult females of *E. finlandicus* were moved to arenas infested with spider mites three days prior to the olfactometer tests.


Fig. 2: Rearing unit for *E. finlandicus*, consisting of a primary bean leaf placed upside down on a water-saturated foam cube inside a plastic box half-filled with water.

## 2.2 Mycorrhizal and non-mycorrhizal bean plants, *Phaseolus vulgaris*

The leaflets used as odor sources in the olfactometer choice tests were taken from mycorrhizal and non-mycorrhizal common bean plants *Phaseolus vulgaris* (var. `Taylor's Horticultural') grown in a walk-in climatic chamber (60 ± 5 % rH, 16:8 h L:D, 23/18° C L:D) in the laboratory (Figure 3). Except of the addition of mycorrhizal inoculum powder to the planting substrate, everything else such as the use of autoclaved substrate, watering, etc. was the same for growing mycorrhizal and non-mycorrhizal plants.

First, seeds of *P. vulgaris* were surface-sterilized in 75% commercial bleach for 3 min, and then thoroughly rinsed with clear water.

Second, 12 seeds were pre-germinated in 1 liter pots filled with perlite, which was previously autoclaved for 20 min at 121 °C. After 7 days, 3 seedlings per pot were transferred to 1 liter pots sterilized with ethanol and filled with an autoclaved 1:1:1 silicate sand/expanded clay/soil substrate mixture.

For generating mycorrhizal bean plants (+M), ~5 g per pot of *Glomus mosseae* inoculum (BEG 12; International Bank of Glomeromycota) was added to the substrate when potting the seedlings (Hoffmann *et al.* 2009). Furthermore, some mycorrhizal soil from previous tests was added to each pot to ease and accelerate the establishment of the mycorrhizal symbiosis between the bean plants and the mycorrhizal fungus (Hoffmann *et al.* 2011b).

For non-mycorrhizal pots (-M), a water filtrate of the mycorrhiza inoculum was added.

Third, both mycorrhizal and non-mycorrhizal bean plants were left to grow for 3 to 5 weeks under standardized environmental conditions (60 ± 5 % rH, 16:8 h L:D, 23/18 °C L:D). The plants were watered once a week with a P-reduced nutrient solution ($Ca(NO_3)_2$ 0.472g/l, $K_2SO_4$ 0.256 g/l, $MgSO_4$ 0.136 g/l, $MoO_3$ 0.07 g, $NH_4NO_3$ 8 mg/l, $Fe_6H_5O_7$ x $3H_2O$ 50 mg/l, $Na_2Bo_4O_7$ X $4H_2O$ 1.3 mg/l, $MnSO_4$ x $4H_2O$ 1.5 mg/l, $ZnSO_4$ x $7H_2O$ 0.6 mg/l, $CuSO_4$ x $5H_2O$ 0.54 mg/l, $Al_2(SO_4)_3$ 0.028 mg/l, $NiSO_4$ x $7H_2O$ 0.028 mg/l, $Co(NO_3)_2$ x $6H_2O$ 0.028 mg/l, $TiO_2$ 0.028 mg/l, $LiCl_2$ 0.014 mg/l, $SnCl_2$ 0.014 mg/l, KJ 0.014 mg/l, and KBr 0.014 mg/l) and twice a week with a $NO_3$ - fertilizer ($KNO_3$ 1.01g/l, $CaNO_3$ 2.36 g/l) to avoid nodulation of rhizobia (Hoffmann *et al.* 2009).

Fourth, each of the three bean plants (+M or –M) was infested with 30 adult females of *T. urticae* 5 days before being used in the olfactometer tests. Clipping the leaves, to be used in the olfactometer tests, from infested plants not later than 5 days after releasing the spider mites ensured that every plant used in the experiments had the same spider mite infestation level because eggs of *T. urticae* need 5-6 days to hatch. For the olfactometer test, the three youngest fully developed trifoliate leaves were detached from the infested bean plants and immediately used.

Fig. 3: Mycorrhizal and non-mycorrhizal bean plants *Phaseolus vulgaris* grown in the walk-in climatic chamber.

## 2.3 Proof of mycorrhizal symbiosis

To quantify the level of mycorrhizal colonization of the roots of bean plants used in the olfactometer test, the method proposed by Vierheilig *et al.* (1998) was applied. After running the olfactometer tests, the roots of the plants were removed from the pots and rinsed with tap water until all remaining soil particles were washed off. The roots were cut into pieces of 2 to 3 cm length and boiled in KOH for 10 min. Then the root samples were thoroughly rinsed with tap water and boiled for 5 min in a 5 % ink-vinegar solution consisting of Schaeffer black ink and household vinegar (5 % acetic acid). Finally, the roots were destained with rinsing tap water and placed in a petridish equipped with gridlines (Giovannetti and Mosse 1980). The percentage of root length colonized by the mycorrhizal fungus (RLC) was estimated according to the method of Newman (1966).

## 2.4 The Y-tube olfactometer

The Y-tube olfactometer used in the experiments (Figure 4) was the same as the one described by Schausberger *et al*. (2012), which is a modification of the olfactometer developed by Sabelis and van de Baan (1983). Three glass tubes (40 mm inner diameter) of equal length (130 mm) were melted together forming a Y-shape. The angle formed between each upper or choice arm and the base arm was 142.5°, the angle in between the choice arms was 75°. The two odor sources, consisting of three mycorrhizal or three non-mycorrhizal trifoliate leaves, were located in two airtight sets of acrylic chambers connected to each of the choice arms. Each set consisted of three tubular acrylic chambers, with a total length of 55 mm and an inner diameter of 35 mm. The outer chamber was filled with activated charcoal to purify the air sucked into the olfactometer; the middle chamber contained the leaves and was connected to the other two chambers with female joints. A male joint, reaching approximately 20 mm inside the choice arm, formed the connection between the set of leaf-containing acrylic chambers and the glass tube. The inner and outer chambers were sealed with gauze, which prevented the predatory mites from entering the chambers or leaf samples and enclosed the charcoal in the outer chamber. A Y-shaped stainless steel wire was placed in equidistance to the glass walls inside the Y-shaped tube and served as a walking path for the predatory mites. The wire started 20 mm inside the bottom of the base arm and was fixed by a vertical 20 mm long extension held in place by a plastic piece placed into a small hole in the upper wall of the Y-tube. The wire branched at the centre of the intersection of the three arms, led through either choice arm and was fixed in the middle of the inner acrylic chambers by inserting it into a small hole in the gauze. To generate an airflow from the odor sources through the choice arms to the end of the base arm towards the predatory mites, which were released on the wire at the bottom end of the base arm, a mini-diaphragm-vacuum pump (Laboport® N 86 KN.18; KNF Neuberger, Freiburg, Germany) was connected to the base arm, sucking air through the olfactometer with a flow rate of 5 liter $min^{-1}$.

During the tests, the olfactometer was placed on a table covered with black cardboard in an air-conditioned room at 25 ± 1 °C. A cold light lamp was placed above the intersection of the Y-tube to prevent any inadvertent bias towards the right or left choice arm caused by the light source.

Fig. 4: Y-tube olfactometer used in the experiments (Schausberger *et al*. 2012).

## 2.5 Choice tests

All gravid females used in the study were randomly withdrawn from their respective rearing units and singly starved in acrylic cages for approximately 12 h prior to running the choice tests. Each cage consisted of a circular hole (1.5 x 0.3 cm) laser-cut into a rectangular piece of acrylic glass with one side closed by a fine nylon mesh (Schausberger 1997). The mites were confined to the cage by closing the other side of the cage with a transparent microscope glide fixed with a binder clip (Figure 5).

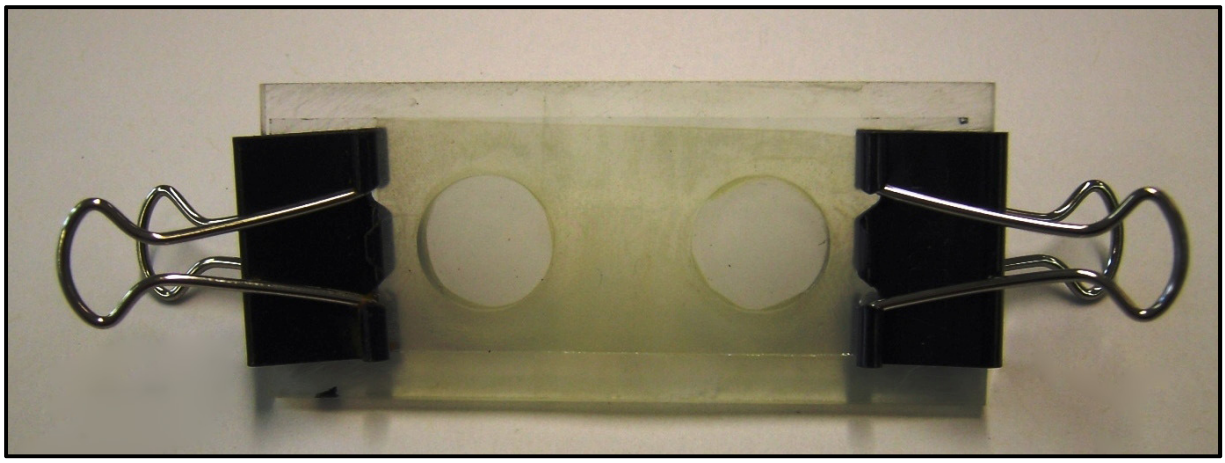

Fig. 5: Acrylic cages used to starve the predators before tests (Schausberger 1997).

Each predatory mite was picked up from the starvation cage, using a moistened camel's hair brush, and released singly at the bottom end of the wire inside the glass tube. Each mite was given a choice between the volatiles emitted by the mycorrhizal and non-mycorrhizal leaf samples and observed for 5 min at maximum whether it decided to move up in one of the two choice arms and reach the end of it, respectively. Predatory mites falling from the wire were discarded from analyses. Predatory mites not reaching the end of a choice arm within 5 min were judged as non-responsive. For each responsive mite, the decision for the +M or –M odor source, the left or right arm and the time needed until reaching the end of an arm were recorded.

The olfactometer experiments were conducted on eight days within a 3.5 weeks period in November 2012. On every olfactometer testing day, five to nine mites of each of the four predatory mite species were tested with the same pair of a mycorrhizal and a non-mycorrhizal leaf sample. Altogether, 60 individuals of *N. californicus* and 47 individuals each of *P. persimilis*, *A. andersoni* and *E. finlandicus* were tested to obtain the data of 40 responsive mites per species. The wire was cleaned with ethanol, using a cotton swab, in

between testing the different species, to avoid any influence by traces left by the mites of another species on the wire. The four species were used in mixed orders on the different days of the experiment. To avoid any inadvertent positional effect, the +M and –M leaf sample pairs were switched between choice arms on every olfactometer testing day, resulting in eight switches between right and left side and eight tested leaf samples throughout the experiments.

## 2.6 Statistical analyses

All statistical analyses were performed with SPSS 15.0 (SPSS Inc. Chicago, IL, USA). In order to determine a preference of *P. persimilis*, *N. californicus*, *A. andersoni* or *E. finlandicus* for the odors of mycorrhizal or non-mycorrhizal bean plants and a possible inadvertent bias towards the left or right hand side of the olfactometer two-tailed binomial tests assuming random choice were performed. To assess the effect of the level of mycorrhization on the choice within each predatory mite species for the emitted odors of mycorrhizal or non-mycorrhizal bean plants bivariate Pearson correlation coefficient analyses were conducted. Separate generalized linear models (GLM) were performed to compare the responsiveness and preference towards the left or right hand side (binomial distribution, logit link) and the mean response times (normal distribution, identity link) among the four species. In order to compare the response times for mycorrhizal or non-mycorrhizal odors of *P. vulgaris* within each predatory mite species, Mann-Whitney *U* tests were performed.

# 3. RESULTS

## 3.1 Root length colonization (RLC)

The roots of all mycorrhizal and non-mycorrhizal bean plants used in the experiments were analyzed for their level of mycorrhizal colonization. The total of eight mycorrhizal plants used in the experiments had an average RLC of 26 % ± 5.5 % (SE). All eight non-mycorrhizal plants were free of mycorrhiza, i.e. had an RLC of 0 %.

## 3.2 Responsiveness

The generalized linear model (GLM, binomial distribution, logit link; posthoc pairwise least significance difference – LSD-tests) showed that *N. californicus* was less responsive than *P. persimilis*, *A. andersoni* and *E. finlandicus* (Wald $\chi^2$ = 8.416, $P$ = 0.038). 33 % (20 out of 60) of tested females of *N. californicus* did not make a decision for either mycorrhizal or non-mycorrhizal bean plants within 5 min. By comparison, only 15 % (7 out of 47) of each *P. persimilis*, *A. andersoni* and *E. finlandicus* were non-responsive (Figure 6).

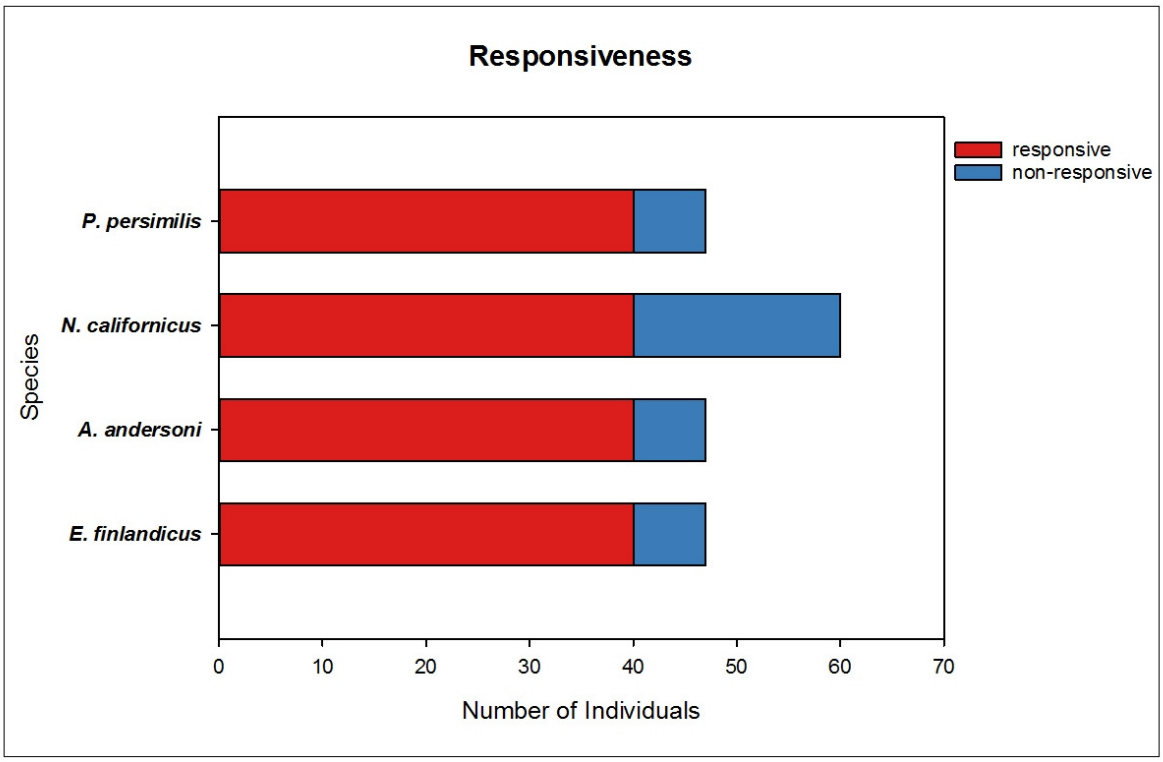


Fig. 6: Responsiveness of the adult females of four species or predatory mites towards odors emitted by mycorrhizal and non-mycorrhizal bean plants (*P. vulgaris*) in the Y-tube olfactometer tests.

## 3.3 Choice of mycorrhizal or non-mycorrhizal bean plants

A total of 40 out of 47 individuals each of *P. persimilis*, *A. andersoni* and *E. finlandicus* responded within 5 min to the odors of either mycorrhizal or non-mycorrhizal bean plants. To obtain 40 responsive mites of *N. californicus* 60 individuals were needed in total. Two-tailed binomial tests within each species, assuming random choice, revealed that only *P. persimilis* preferred mycorrhizal (+M) to non-mycorrhizal (-M) odors ($P < 0.05$), whereas all other three species showed no preference for the odor of either mycorrhizal or non-mycorrhizal plants ($P > 0.05$) (Figure 7). Similarly, cross comparison among species by a generalized linear model (binomial distribution, logit link) showed that the preference for mycorrhizal plants differed among species (Wald $\chi^2 = 10.842$, $P = 0.013$), with *P. persimilis* being different from the other three species (Sequential Sidak; $P < 0.05$. for each pairwise comparison), which were similar to each other (Sequential Sidak; $P > 0.05$ for each pairwise comparison).

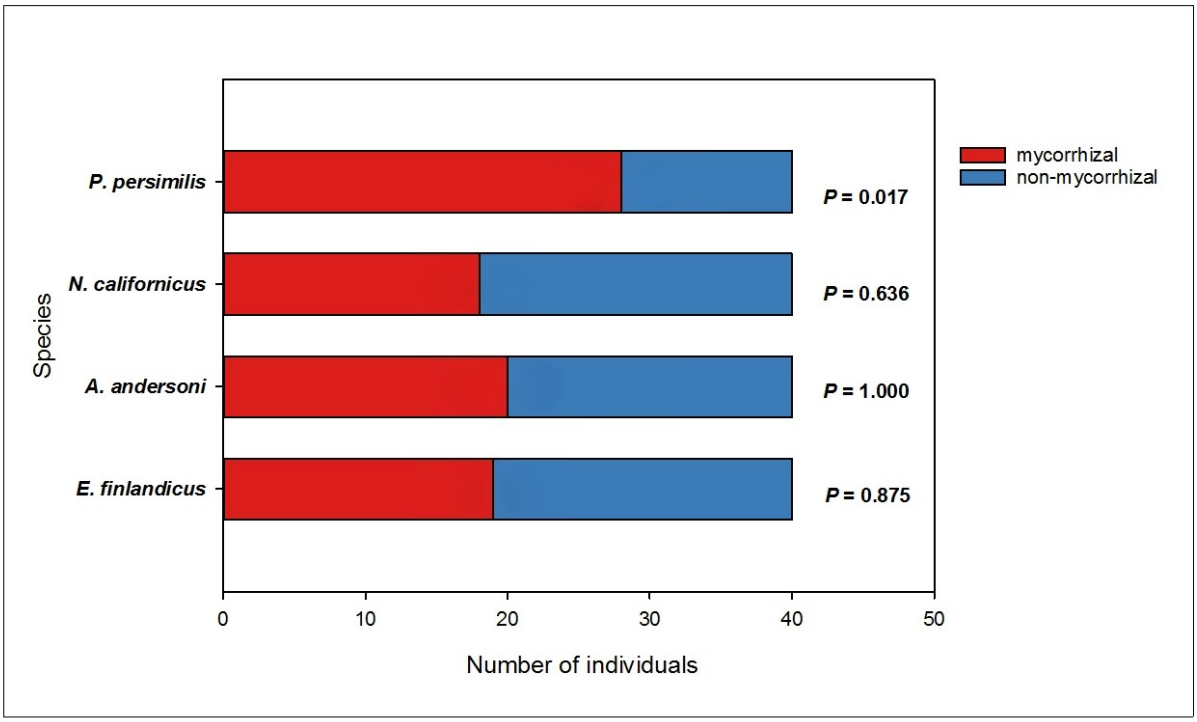


Fig. 7: Choice of the adult females of four species of predatory mites for the odor of mycorrhizal or non-mycorrhizal bean plants (*P. vulgaris*) in the Y-tube olfactometer tests. *P*-values refer to the results of binomial tests within each species, assuming random choice.

No species had a bias towards the left or right hand side of the olfactometer (two-tailed binomial tests within each species, $P > 0.6$; Figure 8). Accordingly, movement towards the left or right hand side of the olfactometer did also not differ among species (GLM; binomial distribution, logit link; Wald $\chi^2 = 1.168$, $P = 0.761$)

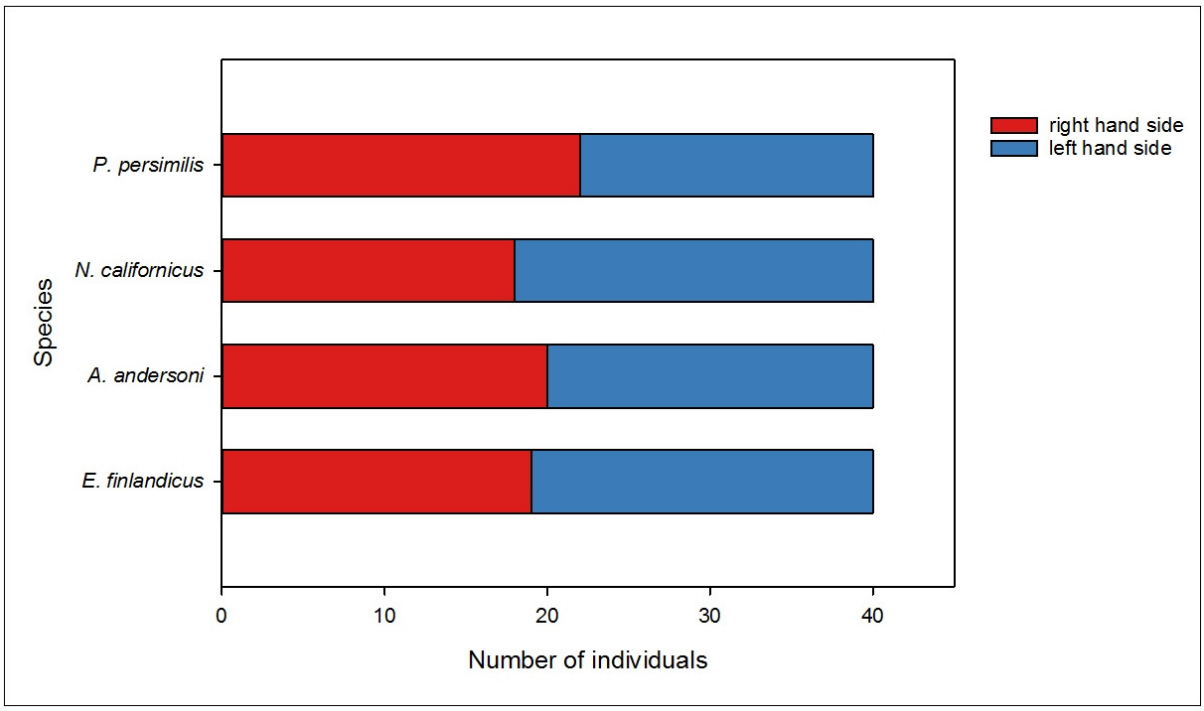


Fig. 8: Decision for the right or left hand side of the adult females of four species of predatory mites for the odor of mycorrhizal or non-mycorrhizal bean plants (*P. vulgaris*) in the Y-tube olfactometer tests.

## 3.4 Correlations between RLC levels and choice

The Pearson correlation coefficients revealed that the level of mycorrhization (RLC) did not have any effect on the decision of any species choosing the odors of mycorrhizal or non-mycorrhizal bean plants (Table 3).

Table 3: Results of bivariate Pearson correlation analyses within each predatory mite species between odor preference for mycorrhizal or non-mycorrhizal bean plants (*P. vulgaris*) and level of mycorrhization of plants used in the Y-tube olfactometer tests.

| Correlations between choice and mycorrhization level | | |
|---|---|---|
| **Species** | **Pearson correlation index** | **(2-tailed) *P*-value** |
| *P. persimilis* | 0.064 | 0.693 |
| *N. californicus* | 0.036 | 0.827 |
| *A. andersoni* | 0.118 | 0.469 |
| *E. finlandicus* | 0.075 | 0.644 |

## 3.5 Response/decision times

Mann-Whitney *U* tests within each species revealed that there was no significant difference in intraspecific response times between the decisions for mycorrhizal (+M) or non-mycorrhizal (-M) bean plant odors (Table 4).

Table 4: Response times (seconds; mean ± SE) of the four predatory mite species for the odor of mycorrhizal (+M) or non-mycorrhizal (-M) bean plants (*P. vulgaris*) in the Y-tube olfactometer tests and results of Mann-Whitney *U* tests.

| Intraspecific response time for +M or –M | | | | | |
|---|---|---|---|---|---|
| **Species** | **Choice** | **N** | **Mean Rank** | **Mean ± SE** | **Asymptotic (2-tailed) *P*-value** |
| *P. persimilis* | +M | 28 | 19.68 | 9.883 | 0.497 |
| | -M | 12 | 22.42 | 18.131 | |
| *N. californicus* | +M | 18 | 23.36 | 14.203 | 0.159 |
| | -M | 22 | 18.16 | 3.829 | |
| *A. andersoni* | +M | 20 | 23.65 | 10.384 | 0.088 |
| | -M | 20 | 17.35 | 10.081 | |
| *E. finlandicus* | +M | 19 | 22.74 | 10.984 | 0.248 |
| | -M | 21 | 18.48 | 8.213 | |

GLM (normal distribution, identity link) showed that *P. persimilis* was the species taking the longest to decide for mycorrhizal or non-mycorrhizal bean plants (GLM; Wald $\chi^2$ = 8.090, $P$ = 0.043; Figure 9). However, posthoc LSD-tests revealed that this was only significant in the pairwise comparison to *N. californicus* ($P$ = 0.006) which was the fastest in response, and marginally significant in comparison to *E. finlandicus* ($P$ = 0.09). All other pairwise comparisons were non-significant ($P$ > 0.1).

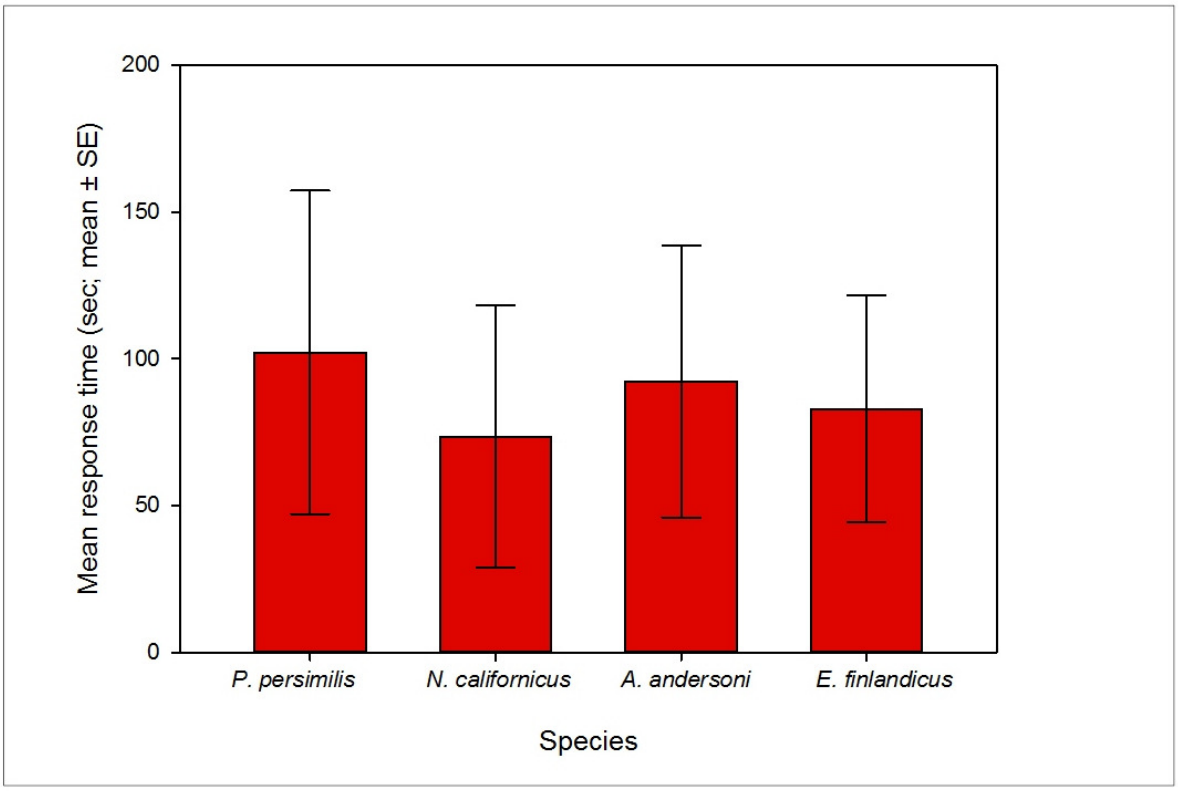


Fig. 9: Response times of all four species to odors emitted by mycorrhizal and non-mycorrhizal bean plants (*P. vulgaris*) in the Y-tube olfactometer tests.

# 4. DISCUSSION

Plants are an important link between below- and aboveground living organisms, as they are primary producers consisting of both belowground (roots) and aboveground (leaves, stems and flowers) organs (Bezemer and van Dam 2005). Some belowground organisms, such as mycorrhizal fungi, form tight associations with plant roots, then called mycorrhiza or mycorrhizal symbiosis, which affect other soil (micro-) organisms as well as the host plants' physiology, chemistry and morphology (Hodge 2000). Mycorrhizal effects cascade up to aboveground plant parts and there influence the plants' interactions with other plant-associated organisms (van der Putten *et al.* 2001). Mycorrhizal symbioses are ubiquitous in the natural environment, generally, but not always, with benefits for both symbiotic partners (Hoeksema *et al.* 2010). The mycorrhizal fungus may have a far-reaching effect on the metabolism of its host plant, through the improved supply with nutrients (Smith and Read 2008), and even influence plant defense mechanisms against pathogens and herbivores, for example through attraction of third trophic level enemies via HIPVs (van der Putten *et al.* 2001). However, since soil communities cannot be monitored that easily, research on HIPVs has mainly focused on aboveground organisms and largely ignoring the fact that above- and belowground plant-associated organisms interact (van der Putten *et al.* 2001).

In my thesis I tried to find out if the aboveground, plant-inhabiting, third trophic level predatory mites *N. californicus*, *A. andersoni* and *E. finlandicus*, all of which are diet generalists, are similarly able to perceive subtle *G. mosseae*-induced changes in HIPVs emitted by the common bean plant *P. vulgaris* infested with the herbivorous two-spotted spider mite *T. urticae*, as is the specialized spider mite predator *P. persimilis* (Hoffmann *et al.* 2011a; Schausberger *et al.* 2012).

The performed olfactometer choice tests revealed that *N. californicus*, *A. andersoni* and *E. finlandicus* do not discriminate HIPVs emitted by *T. urticae*-infested mycorrhizal plants from those emitted by *T. urticae*-infested non-mycorrhizal plants. This result supports my hypothesis that generalist predatory mites with little adaption on *T. urticae* prey are not able to perceive the highly sophisticated mycorrhiza-induced changes in the chemical blend of HIPVs emitted by *T. urticae*-infested bean plants. In contrast, prey-predator co-evolution of *T. urticae* and its prime third trophic level enemy *P. persimilis* apparently selected for such discrimination abilities in this specialist predator, allowing to recognize mycorrhiza-induced

changes in HIPVs (Schausberger *et al.* 2012). Discriminating spider mite-induced HIPVs from mycorrhizal and non-mycorrhizal plants is adaptive for *P. persimilis* because mycorrhizal host plants render the spider mites a more nutritious prey, which consequently increases predator fitness (Hoffmann *et al.* 2011a). Since also the fungus benefits from reduced herbivore pressure on mycorrhizal plants, the interaction between *G. mosseae* and *P. persimilis* may be described as indirectly mutualistic (Hoffmann *et al.* 2011b).

Across different levels of dietary specialization, many predatory mite species are principally able to exploit plant-emitted infochemicals to locate distant herbivore-infested plants (Steidle and van Loon 2003). Nevertheless, the ability of predators to adjust and fine-tune their behavioral response to HIPV blends strongly relies on the dietary specialization of the predators and their prey (Vet and Dicke 1992). During foraging, specialist predators such as *P. persimilis* more likely and more frequently use highly specific cues, such as mycorrhiza-induced changes in HIPVs, which indirectly provide subtle information on the quantity and quality of their main food source, the spider mite *T. urticae*. To the contrary, generalists such as *N. californicus, A. andersoni* and *E. finlandicus*, which are able to feed on various species of mites, insects or pollen, are assumed to make use of general cues providing general information on food availability of different sources but no detailed information on food identity and/or quality (Steidle and van Loon 2003). Therefore, generalists need a broad sensory capacity combined with an ability to switch attentiveness, enabling them to compute the great diversity of perceived infochemicals (Bernays 2001). As the central nervous system of all animals incl. arthropods is limited in its capacity to deal with multiple inputs, generalists are expected to exhibit reduced behavioral efficacy, in response to subtle changes within the infochemicals associated with one and the same food source, as compared to specialists (Bernays 2001). The described general cognitive constraint is a plausible explanation why the generalists used in my experiment did not discriminate between the spider mite-induced odors of mycorrhizal and non-mycorrhizal bean plants, whereas the spider mite specialist did discriminate them.

In my experiments, I only used mycorrhiza-naïve predators, i.e. predators that did not have any experience with spider mite prey feeding on mycorrhizal plants and the HIPVs induced by them on mycorrhizal plants, respectively. Therefore, the ability of *P. persimilis* to discriminate mycorrhizal from non-mycorrhizal odors is obviously innate, whereas *N. californicus, A. andersoni* and *E. finlandicus* lack such an innate ability. However, the generalists might be able to learn discriminating the HIPVs from mycorrhizal and non-

mycorrhizal plants, as generalists rather learn to respond to infochemicals than specialists do (Steidle and van Loon 2003). For example, Gols *et al.* (2012) found that both, the generalist parasitoid *Diadegma fenestrale* and the specialist parasitoid *Diadegma semiclausum* only distinguished between plant volatiles induced by host caterpillars, the diamondback moth *Plutella xylostella*, and those induced by non-host herbivores when the host caterpillars had been feeding on *Brassica oleracea*, the plant on which also the parasitoids had been reared on, but not on other plants. This was likely due to conditioning of the parasitoids to volatiles emitted by their rearing host plant *B. oleracea* (Gols *et al.* 2012). It could thus be that the generalist predatory mites used in my study might possibly learn to perceive subtle changes in HIPVs emitted by mycorrhizal and non-mycorrhizal plants when they are reared on mycorrhizal spider mite-infested bean plants.

Egan and Funk (2006) were the first to provide individual-level evidence of specialist herbivores making host-associated decisions more effectively than generalists and thus enjoy associated fitness advantages. Both larvae and adults of three specialist and generalist conspecific host forms of leaf beetles, *Neochlamisus bebbianae*, were examined. The more specialized beetle host forms exhibited greater efficiency and accuracy than the generalist host forms, both as larvae and adults. This finding suggests that the degree of host specificity is likely to have a major impact on the fitness of *N. bebbianae* and the evolutionary dynamics of ongoing ecological specialization in this species.

Specialists, being able to more accurately locate their optimal prey resource, enjoy a higher relative fitness because their eggs may be laid on plants which provide prey for superior larval growth, thus individual fitness is increased through elevated longevity and fecundity (Egan and Funk 2006).

For herbivores, Bernays (2001) emphasized that specialists may take very good decisions with respect to host quality but that it takes time to make them. This leads us to another finding of my study, the interspecific differences in response times toward the plant odors. The slower response time of *P. persimilis* towards the odors of mycorrhizal and non-mycorrhizal bean plants in comparison to the other three species may have been influenced by the species-specific running speeds but may have been primarily due to the additional time needed for olfactory information processing and decision-making. With extended odorant sampling time the accuracy of odor discrimination increases, which may consequently lead foraging arthropods to a more favorable and more nutritious food source

(Rinberg *et al.* 2006). Therefore, one cost of obtaining valuable information about prey or host plant quality may be the additional time needed to decide between various, simultaneously offered options (Bernays 2001; Chittka *et al.* 2009). Hence, to locate more nutritious prey the specialist predatory mite *P. persimilis* takes longer decision-making times, which may come at the cost of reduced attentiveness to other major life activities such as predator avoidance (Bernays 2001). In the Y-shaped olfactometer *P. persimilis* had to slow down and compare the odors coming from the right and left arms of the tube in order to finally reach the preferred mycorrhizal odor source. In contrast, assuming lacking discrimination ability, *N. californicus*, *A. andersoni* and *E. finlandicus* may have just walked up the wire without stopping at the intersection to evaluate possible differences in the composition of HIPVs, or alternatively or additionally, they may just have run faster than *P. persimilis.*

Regarding decision times, Patiño-Ruiz and Schausberger (2014) observed a similiar phenomenon when examining the possible effects of mycorrhiza on host plant choice of *T. urticae*. Mycorrhiza-experienced spider mites distinguished between the odors of spider mite-uninfested mycorrhizal and non-mycorrhizal bean plants *P. vulgaris*, preferring the former. Due to the fact that mycorrhiza-naïve mites showed no preference for one of the odor sources, Patiño-Ruiz and Schausberger (2014) concluded that the spider mites adaptively learned recognizing mycorrhiza-induced changes in host plant volatiles, guiding them to more nutritious host plants. Similar to my study, mycorrhiza-experienced spider mites took more time to decide for the preferred volatiles than indiscriminative mites, very likely reflecting the costs of the olfactory information and decision making processes.

Up to now, only very few studies have focused on the impact of mycorrhizal fungi on the volatile emissions of their host plants (e.g. Guerreri *et al.* 2004; Leitner *et al.* 2010; Schausberger *et al.* 2012). Schausberger *et al.* (2012) quantified the mycorrhiza-induced changes in the composition of HIPVs of the same variety of *P. vulgaris* employed in my experiments, using proton-transfer mass spectrometry, and revealed that, among other changes, the mycorrhizal plants emitted the two volatile infochemicals β-ocimene and β-caryophyllene at higher amounts than non-mycorrhizal plants, whereas they decreased emission of the constitutively emitted methyl salicylate. As *N. californicus* is attracted to spider mite-infested leaves by linalool and methyl salicylate (Shimoda *et al.* 2005), the reduced emission of methyl salicylate might be one of the reasons why *N. californicus* was not more strongly attracted by the odors of mycorrhizal bean plants, as compared to non-

mycorrhizal plants. However, although predatory mites may be attracted to single compounds of HIPVs induced by spider mite-infestation, it seems that, when making sophisticated choices, they more likely respond to the changed HIPV blends as a whole (van Wijk *et al.* 2010; Schausberger *et al.* 2012).

## 4.1 Conclusion

Herbivorous as well as carnivorous arthropods may exploit volatile chemical information from plants when foraging for food. These chemicals may be produced constitutively by the plant or may be induced by herbivores attacking the plant (Dicke and van Loon 2000). The emission and composition of aboveground HIPVs may not only be affected by events occurring aboveground, but also by belowground processes and organisms such as mycorrhizal fungi (e.g. Guerreri *et al.* 2004; Schausberger *et al.* 2012). Although many generalist predators such as *N. californicus, A. andersoni* and *E. finlandicus* are principally able to exploit HIPVs when foraging for prey, my thesis revealed that these species are not able to discriminate HIPVs emitted by *T. urticae*-infested mycorrhizal plants from *T. urticae*-infested non-mycorrhizal plants. Being able to feed on different species of mites, insects and pollen (McMurtry and Croft 1997), *N. californicus, A. andersoni* and *E. finlandicus* are apparently not able to search for and forage on *T. urticae* as efficiently as is the specialist *P. persimilis*. During life, generalist predators must be able to deal with a large number of food options and associated infochemicals, which is, due to the limited nervous system, traded-off against accuracy in discrimination ability within a given food source (Bernays 2001; Rinberg *et al.* 2006; Chittka *et al.* 2009). In contrast, the *Tetranychus*-specialized *P. persimilis* is able to more accurately identify and precisely locate its optimal prey resource and benefits from recognizing differences in the nutritious value within its preferred prey species, ultimately enhancing its fitness. Co-evolution of *P. persimilis* and its spider mite prey likely resulted in prey-specific adaptations of the predator's sensory and nervous systems, allowing channeling information and enabling the predator to recognize information about prey quality via subtle mycorrhiza-induced changes in HIPVs (Bernays 2001; Hoffmann *et al.* 2011a; Schausberger *et al.* 2012).

# 5. REFERENCES


Bernays, E.A. (2001): Neural limitations in phytophagous insects: implications for diet breadth and evolution of host affiliation. *Annual Review of Entomology* 46:703-727.

Bezemer M., van Dam N.M. (2005): Linking aboveground and belowground interactions via induced plant defenses. *Trends in Ecology and Evolution* 20:617-624.

Brundrett M.C., Piché Y., Peterson R.L. (1985): A developmental study of the early stages in vesicular-arbuscular mycorrhiza formation. *Canadian Journal of Botany* 63:184-194.

Chittka L., Skorupski P., Raine N.E. (2009): Speed-accuracy tradeoffs in animal decision making. *Trends in Ecology & Evolution* 24:400-407.

Croft B.A., McMurtry J.A., Luh H.-K. (1998): Do literature records of predation reflect food specialization and predation types among phytoseiid mites (Acari: Phytoseiidae)? *Experimental & Applied Acarology* 22:467-480.

Currie A. F., Murray P. J., Gange A. C. (2006): Root herbivory by *Tipula paludosa* larvae increases colonization of *Agrostis capillaris* by arbuscular mycorrhizal fungi. *Soil Biology & Biochemistry* 34:1994-1997.

Dicke M., van Beek T. A., Posthumus M. A., Ben Dom N., van Bokhoven H., de Groot A. E. (1990): Isolation and identification of volatile kairomone that affects acarine predator-prey interactions. Involvement of host plants in its production. *Journal of Chemical Ecology* 16:381–396.

Dicke M., Vet L.E.M. (1999): Plant-carnivore interactions: evolutionary and ecological consequences for plant, herbivore and carnivore. In: Olff H., Brown V.K., Drent R. H. (eds): *Herbivores: between plants and predators*. Blackwell Science, Oxford UK, pp. 483-520.

Dicke M., van Loon J.J.A. (2000): Multitrophic effects of herbivore-induced plant volatiles in an evolutionary context. *Entomologia Experimentalis et Applicata* 97:237-249.

Egan S.P., Funk D.J. (2006): Individual advantages to ecological specialization: insights on cognitive constraints from three conspecific taxa. *Proceedings of the Royal Society* B 273:843-848.

Fontana A., Reichelt M., Hempel S., Gershenzon J., Unsicker S.B. (2009): The effects of arbuscular mycorrhizal fungi on direct and indirect defense metabolites of *Plantago lanceolata* L. *Journal of Chemical Ecology* 35:833‑843.

Garriock M.L., Peterson R.L., Ackerley C.A. (1989): Early stages in colonization of *Allium porrum* (leek) roots by the vesicular-arbuscular mycorrhizal fungus *Glomus versiforme*. *New Phytologist* 112:85-92.

Gerson U., Smiley R.L., Ochoa R. (2003): Mites (Acari) for Pest Control. Blackwell Science, Oxford.

Giovannetti M., Mosse B. (1980): An evaluation of techniques for measuring vesicular arbuscular infection in roots. *New Phytologist* 84:489-500.

Gols R., Veenemans C., Potting R.P.J., Smid H.M., Dicke M., Harvey J.A., Bukovinszky T. (2012): Variation in the specificity of plant volatiles and their use by a specialist and a generalist parasitoid. *Animal Behaviour* 83:1231-1242.

Guerreri E., Lingua G., Digilio M.C., Massa N., Berta G. (2004): Do interactions between plant roots and the rhizosphere affect parasitoid behaviour? *Ecological Entomology* 29:753-756.

Helle W., Sabelis M.W. (eds) (1985a): Spider Mites: Their Biology, Natural Enemies and Control. World Crop Pests Vol. 1A. Elsevier, Amsterdam.

Helle W., Sabelis M.W. (eds) (1985b): Spider Mites: Their Biology, Natural Enemies and Control. World Crop Pests Vol. 1B. Elsevier, Amsterdam.

Hodge A. (2000): Microbial ecology of the arbuscular mycorrhiza. *FEMS Microbiology Ecology* 32:91–96.

Hoeksema J., Chaudhary V.B., Gehring C.A., Collins Johnson N., Karst J., Koide R.T., Pringle A., Zabinski C., Bever J.D., Moore J.C., Wilson W.T., Klironomos J.N., Umbanhowar J. (2010): A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. *Ecology Letters* 13:394–407.

Hoffmann D., Vierheilig H., Riegler P., Schausberger P. (2009): Arbuscular mycorrhizal symbiosis increases host plant acceptance and population growth rates of the two-spotted spider mite *Tetranychus urticae*. *Oecologia* 158:663-671.

Hoffmann D., Vierheilig H., Schausberger P. (2011a): Mycorrhiza-induced trophic cascade enhances fitness and population growth of an acarine predator. *Oecologia* 166:141-149.

Hoffmann D., Vierheilig H., Peneder S., Schausberger P. (2011b): Mycorrhiza modulates aboveground tri-trophic interactions to the fitness benefit of its host plant. *Ecological Entomology* 36:574–581.

Hoy M.A. (2011): Agricultural Acarology. Introduction to Integrated Mite Management. CRC Press, Boca Raton.

Hunter M.D., Price P.W. (1992): Playing chutes and ladders—heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. *Ecology* 73:724–732.

Isobe K., Tsuboki Y. (1999): Study on the utilization of arbuscular mycorrhizal fungi in kidney bean cultivation - differences in kidney bean growth resulting from the infection of six species of arbuscular mycorrhizal fungi. *Japanese Journal of Crop Science* 68:112-117.

Koricheva J., Gange A.C., Jones T. (2009): Effects of mycorrhizal fungi on insect herbivores: a meta-analysis. *Ecology* 90:2088-2097.

Kostiainen T., Hoy M.A. (1994): Egg-harvesting allows large scale rearing of *Amblyseius finlandicus* (Acari: Phytoseiidae) in the laboratory. *Experimental & Applied Acarology* 18:155–165.

Koveos D.S., Kouloussis N.A., Broufas G.D. (1995): Olfactory responses of the predatory mite *Amblyseius andersoni* Chant (Acari, Phytoseiidae) to bean plants infested by the spider mite *Tetranychus urticae* Koch (Acari, Tetranychidae). *Journal of Applied Entomology* 119:615-619.

Leitner M., Kaiser R., Hause B., Boland W., Mithöfer A. (2010): Does mycorrhization influence herbivore-induced volatile emission in *Medicago truncatula*? *Mycorrhiza* 20:89-101.

McMurtry J.A., Croft B.A. (1997): Life styles of phytoseid mites and their roles in biological control. *Annual Review of Entomology* 42:291-321.

McMurtry J.A., De Moraes G.J., Sourassou N.F. (2013): Revision of the lifestyles of phytoseiid mites (Acari: Phytoseiidae) and implications for biological control strategies. *Systematic & Applied Acarology* 18:297-320.

Mumm R., Dicke M. (2010): Variation in natural plant products and the attraction of bodyguards for indirect plant defense. *Canadian Journal of Zoology* 88:628-667.

Newman E.I. (1966): A method of estimating total length of root in a sample. *Journal of Applied Ecology* 3:139-140.

Ortas I., Akpinar C. (2006): Response of kidney bean to arbuscular mycorrhizal inoculation and mycorrhizal dependency in P and Zn deficient soils. *Acta Agriculturae Scandinavica*, B 56:101-109.

Patiño-Ruiz J.D., Schausberger P. (2014): Spider mites adaptively learn recognizing mycorrhiza-induced changes in host plant volatiles. *Experimental & Applied Acarology* 64:455-463.

Pozzebon A., Duso C. (2008) Grape downy mildew *Plasmopora viticola*, an alternative food for generalist predatory mites occurring in vineyards. *Biological Control* 45:441–449.

Price P.W., Bouton C.E., Gross P., McPheron B.A., Thompson J.N., Weis A.E. (1980): Interactions among three trophic levels: Influence of plants on interactions between insect herbivores and natural enemies. *Annual Review of Ecology and Systematics* 11:41-65.

Read D.J., Perez-Moreno J. (2003): Mycorrhizas and nutrient cycling in ecosystems – a journey towards relevance? *New Phytologist* 157:475-492.

Reddy G. V. P., Holopainen J. K., Guerrero A. (2002): Olfactory responses of *Plutella xylostella* natural enemies to host pheromone, larval frass, and green leaf cabbage volatiles. *Journal of Chemical Ecology* 28:131-143.

Rinberg D., Koulakov A., Gelperin A. (2006): Speed-accuracy tradeoff in olfaction. *Neuron* 51:351-358.

Roda A., Nyrop J., Dicke M., English-Loeb G. (2000): Trichomes and spider-mite webbing protect predatory mite eggs from intraguild predation. *Oecologia* 125:429–436.

Rosewarne G.M., Barker S. J., Smith S. E. (1997): Production of near-synchronous fungal colonization in tomato for developmental and molecular analyses of mycorrhiza. *Mycological Research* 101:966-970.

Sabelis M.W., van de Baan H.E. (1983): Location of distant spidermite colonies by phytoseiid predators: demonstration of specific kairomones emitted by *Tetranychus urticae* and *Panonychus ulmi*. *Entomologia Experimentalis et Applicata* 33:303–314.

Sabelis M.W., Bakker F.M. (1992): How predatory mites cope with the webs of their tetranychid prey: a functional view on dorsal chaetotaxy in the Phytoseiidae. *Experimental & Applied Acarology* 16:203–225.

Saito Y. (1985): Life types of spider mites. In: Helle W., Sabelis M.W. (eds) (1985a): *Spider Mites: Their Biology, Natural Enemies and Control*. World Crop Pests Vol. 1A. Elsevier, Amsterdam, pp. 253-264.

Schmidt G. (1976): Der Einfluss der von den Beutetieren hinterlassenen Spuren auf Suchverhalten und Sucherfolg von *Phytoseiulus persimilis* Athias-Henriot (Acarina: Phytoseiidae). *Zeitschrift für Angewandte Entomologie* 82:216–218.

Schausberger P. (1992): Vergleichende Untersuchungen über den Einfluß unterschiedlicher Nahrung auf die Präimaginalentwicklung und die Reproduktion von *Amblyseius aberrans* Oud. und *Amblyseius finlandicus* Oud. (Acarina, Phytoseiidae). *Journal of Applied Entomology* 113: 476–486.

Schausberger P. (1997): Inter- and intraspecific predation on immatures by adult females in *Euseius finlandicus*, *Typhlodromus pyri* and *Kampimodromus aberrans* (Acari: Phytoseiidae). *Experimental & Applied Acarology* 21:131–150.

Schausberger P. (1998) Survival, development and fecundity in *Euseius finlandicus*, *Typhlodromus pyri* and *Kampimodromus aberrans* feeding on the San Jose Scale (*Quadraspidiotus perniciosus*). *Journal of Applied Entomology* 122:53–56.

Schausberger P., Peneder S., Jürschik S., Hoffmann D. (2012): Mycorrhiza changes plant volatiles to attract spider mite enemies. *Functional Ecology* 26:441-449.

Shimoda T., Ozawa R., Sano K., Yano E., Takabayashi J. (2005): The involvement of volatile infochemicals from spider mites and from food-plants in prey location of the generalist predatory mite *Neoseiulus californicus*. *Journal of Chemical Ecology* 31:2019-2032.

Shimoda T., Kishimoto H., Takabayashi J., Amano H., Dicke M. (2009): Comparison of thread-cutting behavior in three specialist predatory mites to cope with complex webs of *Tetranychus* spider mites. *Experimental & Applied Acarology* 47:111-120.

Smith S.E., Read D.J. (2008): Mycorrhizal Symbiosis, 3rd edition. Elsevier, Amsterdam.

Steidle J.L.M., van Loon J.J.A. (2003): Dietary specialization and infochemical use in carnivorous arthropods: testing a concept. *Entomologia Experimentalis et Applicata* 108:133-148.

Sun X., Yin S., Li B., Sun X. G., Yin S. Y., Li B. (2002): Studies on the interrelationship among *Oligonychus ununguis* host plants and *Amblyseius finlandicus*. *Scientia Silvae Sinicae* 38:76-81.

Tomczyk A., Kropczynska D. (1985): Effects on the Host Plant. In: Helle W., Sabelis M.W. (eds) (1985a): *Spider Mites: Their Biology, Natural Enemies and Control*. World Crop Pests Vol. 1A. Elsevier, Amsterdam, pp. 317-329.

van der Putten W.H., Vet L.E.M., Harvey J.A, Wäckers F.L. (2001): Linking above- and belowground multitrophic interactions of plants, herbivores, pathogens and their antagonists. *Trends in Ecology & Evolution* 16:547–554.

van Leeuwen T., Vontas J., Tsagkarakou A., Dermauw W., Tirry L. (2010): Acaricide resistance mechanisms in the two-spotted spider mite *Tetranychus urticae* and other important Acari: A review. *Insect Biochemistry and Molecular Biology* 40:563-572.

van Wijk M., de Bruijn P.J.A, Sabelis M.W. (2010): The predatory mite *Phytoseiulus persimilis* does not perceive odor mixtures as strictly elemental objects. *Journal of Chemical Ecology* 36:1211-1225.

Vet L.E.M, Dicke M. (1992): Ecology of infochemical use by natural enemies in a tritrophic context. *Annual Review of Entomology* 37:141-173.

Vierheilig H., Coughlan A.P., Wyss U., Piché Y. (1998): Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. *Applied and Environmental Microbiology* 64:5004-5007.

Wamberg C., Christensen S., Jakobsen I. (2003): Interaction between foliar-feeding insects, mycorrhizal fungi, and rhizosphere protozoa on pea plants. *Pedobiologia* 47:281-287.

Zhang Z.-Q., Sanderson J.P., Nyrop J.P. (1992): Foraging time and spatial patterns of predation in experimental populations - A comparative study of three mite predator-prey systems (Acari: Phytoseiidae, Tetranychidae). *Oecologia* 90:185-196.

# 6. LIST OF FIGURES



# 7. LIST OF TABLES

# 8. ABSTRACT

Some belowground micro-organisms, such as arbuscular mycorrhizal fungi (AMF), form tight associations with plant roots, which may have far-reaching effects upon plant metabolism. AMF may influence plant defense responses both above- and belowground, a fact that was neglected in the past. Only recently, research has begun to consider that above- and belowground organisms may interact across trophic levels of multi-trophic food webs. Previous studies revealed that (1) the herbivorous two-spotted spider mite *Tetranychus urticae* performs better on mycorrhizal than non-mycorrhizal bean plants; (2) mycorrhizal symbiosis (AM) between the AMF *Glomus mosseae* and the common bean plant *Phaseolus vulgaris* cascades up the food web and also influences aboveground indirect defense responses of the plant, recruiting the spider mites' prime natural enemy, the predatory mite *Phytoseiulus persimilis*; (3) this specialist predator is able to recognize mycorrhiza-induced changes in the composition of herbivore induced plant volatiles (HIPVs), guiding it to more nutritious spider mite prey. In my thesis, I asked whether recognition of subtle mycorrhiza-induced changes in HIPVs emitted by spider mite-infested plants is due to co-evolution between the specialized spider mite predator *P. persimilis* and its prey. Accordingly, I hypothesized that more generalized third trophic level predatory mites, such as *Neoseiulus californicus*, *Amblyseius andersoni* and *Euseius finlandicus*, are incapable of discriminating HIPVs emitted by *T. urticae*-infested mycorrhizal plants from *T. urticae*-infested non-mycorrhizal plants. To address this hypothesis, I performed Y-tube olfactometer experiments comparing the response of the spider mite specialist *P. persimilis* and the diet generalists *N. californicus*, *A. andersoni* and *E. finlandicus* to simultaneously offered odors from mycorrhizal and non-mycorrhizal spider mite-infested bean plants. Only *P. persimilis* but none of the diet generalists preferred the HIPVs of mycorrhizal plants to those of non-mycorrhizal plants. Additionally, *P. persimilis* took longer decision times than the generalists, probably reflecting the costs of decision-making as compared to being indiscriminate. Altogether, these results support the hypothesis that, in contrast to the spider mite specialist *P. persimilis*, generalist predatory mites with little adaption on *T. urticae* prey have not been selected to innately recognize mycorrhiza-induced changes in the chemical blend of HIPVs emitted by *T. urticae*-infested bean plants.

# 9. KURZZUSAMMENFASSUNG

Manche unterirdisch lebende Mikroorganismen, wie zum Beispiel Arbuskuläre Mykorrhizapilze (AMF), gehen enge symbiotische Verbindungen mit Pflanzenwurzeln ein, und können dadurch weitreichende Auswirkungen auf den Stoffwechsel der Pflanze haben. AMF können sowohl ober- als auch unterirdische Abwehrreaktionen der Pflanze gegen Fressfeinde und Pathogene beeinflussen, eine Tatsache, die lange außer Acht gelassen wurde. Erst seit Kurzem wird in der Forschung berücksichtigt, dass ober- und unterirdische, mit Pflanzen assoziierte Organismen über verschiedene trophische Level in vielschichtigen Nahrungsnetzen interagieren können. Frühere Studien belegen, dass (1) die herbivore Gemeine Spinnmilbe *Tetranychus urticae* auf mykorrhizierten Bohnenpflanzen besser gedeiht als auf nicht mykorrhizierten; (2) die Symbiose zwischen dem AMF *Glomus mosseae* und der Gartenbohne *Phaseolus vulgaris* trophische Kaskaden auslöst, die oberirdisch den wichtigsten natürlichen Fressfeind der Spinnmilben, die Raubmilbe *Phytoseiulus persimilis*, positiv beeinflussen; (3) dieser, auf *T. urticae* spezialisierte, Räuber Mykorrhiza-induzierte Veränderungen in der Zusammensetzung der von den Herbivoren induzierten Pflanzendüfte (*herbivore-induced plant volatiles* – HIPVs) wahrnehmen kann, welche ihn folglich zu nahrhafteren Beutetieren führen. Grundlage meiner Diplomarbeit war die Annahme, dass die Wahrnehmung und Erkennung der subtilen Mykorrhiza-induzierten Veränderungen in den HIPVs von spinnmilbeninfizierten Pflanzen auf die Koevolution von *P. persimilis* und ihrer Beute zurückzuführen ist. Demnach habe ich die Hypothese aufgestellt, dass omnivore Raubmilben mit einem breiten Nahrungsspektrum, wie z.B. *Neoseiulus californicus*, *Amblyseius andersoni* und *Euseius finlandicus*, im Gegensatz zu *P. persimilis* nicht in der Lage sind, die Unterschiede zwischen den HIPVs von Spinnmilben-infizierten mykorrhizierten und nicht mykorrhizierten Bohnenpflanzen zu erkennen. Zur Überprüfung dieser Hypothese, habe ich ein Wahl-Experiment mit einem Y-förmigen Olfaktometer durchgeführt, in welchem die Reaktionen des Spinnmilbenspezialisten *P. persimilis* und der omnivoren Raubmilben *N. californicus*, *A. andersoni* and *E. finlandicus* auf die simultan angebotenen Düfte von mykorrhizierten und nicht mykorrhizierten spinnmilbeninfizierten Bohnenpflanzen getestet wurden. Nur *P. persimilis* erkannte und bevorzugte die HIPVs von mykorrhizierten Pflanzen, die omnivoren Räuber zeigten keine Präferenz. Darüber hinaus brauchte *P. persimilis* länger für die Entscheidungsfindung als die anderen Raubmilbenarten. Dies reflektiert höchstwahrscheinlich die ökologischen Kosten des Vergleichens und der Entscheidungsfindung, im Vergleich zur Indifferenz.

Zusammenfassend bestätigen meine Resultate die These, dass omnivore Raubmilben, die im Gegensatz zu *P. persimilis* nicht auf *T. urticae* als Beute spezialisiert sind, aufgrund fehlender enger Koevolution nicht dahin gehend selektiert wurden, subtile Mykorrhiza-induzierte Veränderungen in den HIPVs von spinnmilbeninfizierten Pflanzen wahrzunehmen.

# 10. CURRICULUM VITAE

## Personal data

| | |
|---|---|
| **Name:** | Patricia Erler |
| **Date of Birth:** | February 16th, 1987 |
| **Place of Birth:** | Innsbruck, Austria |
| **Nationality:** | Austrian |
| **Marital Status:** | Single |

## Education

| | |
|---|---|
| Mar. 2011 – Nov. 2014 | Master's program Agricultural Biology,<br>University of Natural Resources and Life Sciences, Vienna |
| Oct. 2007 – Mar. 2011 | Bachelor's program Agricultural Sciences,<br>University of Natural Resources and Life Sciences, Vienna |
| Sept. 2006 – Aug. 2007 | Aupair in Limerick, Ireland |
| Sept. 2001 – Jun. 2006 | Higher Vocational School for Service Industries Management<br>(HBLA für wirtschaftliche Berufe), Innsbruck |

## Professional Experience

| | |
|---|---|
| Jan. 2009 – | Kommunalkredit Austria, Vienna. Part time assistance of the marketing department |
| Jul. 2009 – Sept. 2009 | Agricultural internship, Agricultural Technical School Zwettl, Austria |
| Jan. 2008 – Sept. 2014 | Part time babysitting |

## Qualifications

| | |
|---|---|
| *Language skills* | German (first language)<br>English (fluent)<br>Italian (basic) |
| *Computer literacy* | Experienced handling of MS Office & Windows |