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MASTER THESIS

Effects of mycorrhiza on long distance attraction of spider mites to bean plants

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Specially dedicated to my family who supported every of my decisions
during my entire life.

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ABSTRACT

The root symbionts arbuscular mycorrhiza fungi (AMF) tend to change various attributes of their host plants, commonly improving plant health and constitution. The mycorrhiza-induced changes in plant performance may also have an influence on other plant-associated organisms such as aboveground living herbivores and their natural enemies. These influences are mainly caused by morphological, physiological and biochemical changes of the plant resulting from their symbiosis with the AMF. The effects of arbuscular mycorrhizal symbiosis (AM) on aboveground herbivores are highly variable, ranging from positive to neutral to negative. In the case of the leaf-sucking two-spotted spider mite *Tetranychus urticae* feeding on common bean plants *Phaseolus vulgaris*, previous studies revealed that AM enhances the fitness of the spider mites and changes the plant-emitted volatiles to more strongly attract the natural enemies of the spider mites, the predatory mite *Phytoseiulus persimilis*. However, there is no current information that can tell of the effects of AM on long-distance attraction of the spider mites via plant-emitted volatiles. We took on the task to determine if AM has an influence on the foraging behavior of *T. urticae* when it comes to choose between bean plants to colonize and feed. Y-tube olfactometer tests were performed in order to analyze the preference of mycorrhiza-naïve and -experienced spider mites to volatiles of bean plants, inoculated with the AMF *Glomus mosseae* or not and infested with the spider mites or not. Experienced mites were those previously reared on mycorrhizal bean plants. The olfactometer experiments revealed that AM does have an effect on the host plant choice of the spider mites. Mycorrhiza-experienced mites showed a preference towards the volatiles of AMF-inoculated non-infested plants. In contrast, mycorrhiza-naïve mites exposed to volatiles of non-infested mycorrhizal and non-mycorrhizal plants and mycorrhiza-naïve and -experienced mites exposed to volatiles of spider mite-infested mycorrhizal and non-mycorrhizal plants did not have a preference. These results provide evidence of adaptive learning because experience allowed the mites to select those host plants that were the most favorable to their fitness.

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1. INTRODUCTION

All around the planet we find all types of interactions between different organisms, plants are no exception. For example, most plants can create a symbiotic relation with fungi such as arbuscular mycorrhizal fungi (AMF), which are common organisms present in the soils of many ecosystems (Allen 1996). The symbiotic association between the fungi and the plant roots is commonly called arbuscular mycorrhiza (AM). In most cases this relation turns into a positive outcome for both the host plant and the AMF, and is thus to be considered a mutualistic relation (Smith & Read 2008). However, not only the plant and the fungus are affected by this symbiosis. Plant-associated organisms above- and belowground such as herbivores (Koricheva *et al.* 2009), root-feeding insects (Gange 2001) and 3rd trophic level organisms such as predators (Schausberger *et al.* 2012) and parasitoids (Guerrieri *et al.* 2004), or pollinators (Wolfe *et al.* 2005) are also affected by this interaction between the plant and the AMF.

Multi-trophic level interactions are difficult to assess and can be highly variable due to the influence of external biotic and abiotic factors (van der Heijden & Sanders 2002). There are many studies on the interactions between the fungi and the plant and between the plant and herbivores, respectively, but studies on the plant-mediated interactions between aboveground plant-associated organisms such as herbivorous insects and mites and belowground micro-organisms such as AMF are limited (van der Putten *et al.* 2001). Most of the studies or theories on plant-insect interactions are dealing with bitrophic systems and are mainly based on aboveground organisms. The latter is probably due to the fact that the belowground is more difficult to study than the aboveground.

1.1. The study organisms and their interactions

1.1.1. Arbuscular mycorrhiza

The associations between plant roots and AMF are one of the most important and ubiquitous symbioses on earth (Allen 1996). The majority of plants form symbiosis with soil-borne fungi, with AMF being the most common. Mycorrhizal symbiosis occurs in ~80% of the world's flowering plant species, mostly forbs and grasses, and is formed by ~150 different fungal species (Allen 1996, Koricheva *et al.* 2009). Both arbuscular

(endo-) mycorrhiza and ectomycorrhiza act in a similar manner, providing the host plant with mineral nutrients and water through their hyphal network in return for carbohydrates (Allen 1996, Koricheva *et al.* 2009). One feature of the AMF is the hyphal connection without cross walls (also called septa which divide the hyphae into cells) that connects the soil with the root cortex. The hyphae penetrate the roots and grow intercellularly to their inner cortical layers. Then they penetrate individual cells and form arbuscules from the coils, with the arbuscule being an important point of contact for the exchange of resources between the plant and the fungi. Phosphorous (P) is concentrated in these parts and also carbon (C) transfer appears to be mainly concentrated in the arbuscules (Allen 1996). One of the most important roles of the AM is the improvement of nutrient uptake (Smith & Read 2008, Koricheva *et al.* 2009). Nitrogen (N) and P in the soil can be found locked in organic macromolecules, which can only be accessible to decomposers. Some classes of mycorrhizal fungi can mobilize these elements from the primary source or their intermediates, providing the plant with access to these nutrients before they are re-immobilized by micro-organisms. In general, these fungi increase the efficiency with which the plants get and recycle the nutrients (Read & Pérez-Moreno 2003). This increase in nutrient uptake generally leads to a better nourished plant. It has been shown that AM specially increases the P uptake from the soil thus causing a higher P content in aboveground plant tissues (Read 1998). Moreover, it has also been proven that AM is capable of amending the uptake of the macro-element N, allowing the plant to access more easily to organic N sources (necromass) in the soil (Hodge *et al.* 2001, Read & Pérez-Moreno 2003). AM can also serve as soil C sink especially under conditions of high fertility and soil humidity. This C sink feature of the AM allows the plant to access C directly from the soil without going through all the decomposition processes (Kapulnik & Douds 2000). It is not only the plant that gets benefits from this symbiosis but also the AMF benefits by obtaining C in return in the form of carbohydrates from the plant for the exchange of nutrients. Between 4 to 20% of the photosynthates are transferred to the fungus and used for production of both vegetative and reproductive structures and for respiration to support growth and maintenance, including nutrient uptake (Smith & Read 2008). Allen (1996) mentions that under high levels of CO₂ the C uptake from the plant should increase, making the AM more important since nutrients become more limiting. External conditions - environmental stress such as poor soil conditions and herbivory - could cause a decrease in the plant's photosynthate production, resulting in a plant unable to

produce enough photosynthate to support the AMF causing a switch from symbiosis to parasitism (e.g. Fontana *et al.* 2009).

1.1.1.1. The arbuscular mycorrhizal fungus *Glomus mosseae*

In the interaction between plants and mycorrhizal fungi, first of all the type of fungus plays a decisive role, i.e. whether it is an AMF, where the symbiosis is then called endomycorrhiza, or an ectomycorrhizal fungus. *Glomus mosseae* Nicol. & Gerd, the fungus employed in this study, is a ubiquitous AMF, which acts as a root symbiont of numerous vascular plants (Giovannetti *et al.* 1993). Regarding plant-mediated interactions with aboveground herbivores, *G. mosseae* is, for example, known to have an effect on host plant choice and life history performance of the herbivorous two-spotted spider mite *Tetranychus urticae* Koch, by changing the nutrient status of its host plant common bean *Phaseolus vulgaris* L. (Hoffmann *et al.* 2009). Hoffmann *et al.* (2011) furthermore showed that *G. mosseae* modulates the tritrophic interactions between the host plant (*P. vulgaris*), the herbivore (*T. urticae*) and the natural enemy of the herbivores, the predatory mite *Phytoseiulus persimilis* Athias-Henriot, to the benefit of the plant. The AM helped the plant to have a higher seed production, an increase in plant tolerance to herbivore attack and a stronger attraction of the natural enemies of the spider mites to the plant, altogether lessening the damage caused by the herbivores. The presence of the predatory mites resulted in higher mycorrhizal colonization levels, probably due to a decrease in herbivore pressure, assuming that more carbohydrates would be allocated to the plant roots allowing a better establishment of the AM (Hoffmann *et al.* 2011). AMF colonized plants tended to have higher host plant quality for the spider mites (Hoffmann *et al.* 2009), which cascaded up to the next trophic level, the predators and parasitoids, resulting in higher predator densities due to a higher prey quality (Hoffmann *et al.* 2011). However, in other systems mycorrhiza-induced changes in plant growth and chemistry may also result in a negative effect on 3rd trophic level organisms such as parasitoids. The phytotoxins produced by the plant as defense against herbivores usually accumulate in tissues such as body fat and hemolymph affecting developing parasitoids that feed from the host herbivore (e.g. Soler *et al.* 2012).

1.1.2. The mite *Tetranychus urticae* (Acari: Tetranychidae)

The two-spotted spider mite *Tetranychus urticae* belongs to the spider mite family Tetranychidae. It is a polyphagous pest feeding on the plant surface by penetrating the

leaf up to a depth of 70-120 μm with their stylets and sucking on the cell content (Tomczyk & Kropczynska 1985). *T. urticae* is globally distributed and has major economic importance in agriculture (Helle & Sabelis 1985) with over 900 host plant species recorded (e.g. Bolland *et al.* 1998, Hoffmann & Schausberger 2012). This species shows relatively high variability in its morphology, physiology, sex ratio, etc. (e.g. Tsagkarakou *et al.* 1997).

Adult females of *T. urticae* are ~ 0.4 mm long. Every mobile life stage produces a thread of silk when walking (Helle & Sabelis 1985). The life cycle of the spider mite consists of the egg, larva, two nymphal stages (protonymph and deutonymph) and the adult. Reproduction by the spider mites is extremely sensitive to intrinsic factors such as mite strain and level of inbreeding, colony density, and age of female and of population, female's fertilization status, quality of mate, duration of insemination and various behavioral aspects. Similarly, reproduction also depends on extrinsic factors such as temperature, humidity, light, level of predation, intra- and interspecific competition, quantity, quality and timing of pesticides, and various features of the host plants such as strain, plant and soil nutrition and plant age (Wrensch 1985). Within the family Tetranychidae, the highest intrinsic rate of natural increase (r_m) is found in the Tetranychinae. *Tetranychus* species such as *T. urticae* appear to be the most prolific with an oviposition rate of 10 eggs per day when females are 5 days old (Gutierrez & Helle 1985), with a mean of 103.3 eggs per female for its whole life (Sabelis 1985).

The spider mites can reach their food sources in different ways, with passive dispersal by wind being the most common form of long distance dispersal for such a small organism. Ambulatory dispersal, i.e. walking or running, is used for local movements on a leaf or on a plant between leaves or between plants. Yet another form is sudden downward vertical dispersal, also called voluntary falling, in which odor detection is involved (Ohzora & Yano 2011).

1.1.2.1. Interactions between spider mites and their host plants

Spider mites use their host plants as a food source and site for reproduction, which commonly results in a detriment for plant fitness. Passive wind dispersal being the most common way of dispersal for the mites, the process of local host plant selection should be viewed as host plant acceptance and not host plant finding (Sabelis 1985). When a spider mite reaches a suitable host plant and begins to feed, it also starts to form webs,

which are then used for protecting the eggs by placing them on the threads (Gerson 1985). Once the spider mites start feeding, it is most common that they do it on the underside of the leaves; however, some species reside and feed on both sides of the leaf (Tomczyk & Kropczynska 1985).

Some spider mites have the capability to learn to differentiate between different qualities of feeding and reproduction sites. Experienced mites then often prefer the site at which they achieve a higher fitness. Accordingly, when having a choice, *T. urticae* experienced with various host plants should select those plants that offer the best prospects for their reproductive success (Dicke 1986). This phenomenon is referred to as adaptive learning (Papaj & Lewis 1993, Egas & Sabelis 2001). Egas & Sabelis (2001) documented this behavior in *T. urticae* by first letting the mites experience cucumber and tomato plants. Experience with cucumber resulted subsequently in a clear preference for the cucumber plants. The reproductive output of the mites was higher on cucumber plants, suggesting that learning was adaptive. The mites did not perceive the quality of the plant at the first instant but they needed a feeding experience on the plants to come up with a preference.

1.1.3. Interactions between arbuscular mycorrhiza and the herbivores

1.1.3.1. Effects of arbuscular mycorrhiza on aboveground herbivores

The effects of AMF on aboveground herbivores are highly variable ranging from positive to neutral to negative (e.g. Gehring & Whitham 2002) and influenced by several biotic and abiotic factors as well as plant and fungal genotypes (Johnson *et al.* 1997). In the absence of herbivory, the mycorrhizal symbiosis often results in an enhancement of plant health due to a direct connection created by the AMF of the plant roots with the soil resources, allowing the plant to increase its nutrient uptake. Under relatively benign conditions, herbivory may have no effects on AM, but as plant health suffers a detriment due to herbivore action the AM may as well be affected. The effects of mycorrhiza on the herbivores also vary with the type, whether it is arbuscular mycorrhiza (AM) or ectomycorrhiza (EM), and species of mycorrhizal fungus. Moreover, different types of herbivores may be differently affected by mycorrhizal fungi (Gehring & Whitham 2002, Koricheva *et al.* 2009). Overall, generalist herbivores tend to be more negatively affected than specialists. Since mycorrhiza may alter the concentrations of defensive chemicals within the host plant tissue, such changes might

affect specialized herbivores, which have sophisticated mechanisms for detoxification or sequestration, differently than polyphagous ones, which usually have a less well-developed ability to handle the defensive compounds from their host plants. Leaf chewers are more likely negatively affected while sap feeders and their allies are more likely positively affected by mycorrhiza (Gehring & Whitham 2002). Plant chemical defense-related changes induced by mycorrhiza are likely to affect chewing insects more strongly than sucking insects because the chewing insects consume plant tissues, where defensive toxins are stored, while the toxins are present in lower concentrations in the phloem sap. Mycorrhiza can alter the plant's allocation to defense by increasing or improving the nutritional status of the plant, which would lead to a higher primary productivity, increasing the resources for the plant to use in biosynthesis or defensive metabolites such as volatile organic compounds (VOCs) (Fontana *et al.* 2009). Mycorrhiza could also alter the plant C:N ratios, which would allow an increased investment in C-based anti-herbivore defenses reducing herbivore performance (Gehring & Whitham 2002). An improvement in N-based chemical defense may occur due to an increase in N uptake. This could also result in an increase in plant tolerance to herbivory when a higher N uptake would promote a more vigorous shoot and root growth, allowing the plant to better tolerate herbivore attacks without the loss of productivity. However, it is also possible that this type of symbiosis causes an increase of herbivore attacks, mainly because the C allocated to support the AM, cannot be used for defense (Kapulnik & Douds 2000). In general, plants under attack by herbivores may basically defend themselves through the use of two mechanisms, both of which may be affected by mycorrhiza: (1) direct mechanisms, which involve the development of morphological structures (e.g. glands, thorns, trichomes), or chemical compounds that are detrimental to the herbivores (van der Putten *et al.* 2001); (2) indirect mechanisms, which involve the production of secondary metabolites or plant volatiles, which will attract the natural enemies of the herbivores like predators or parasitoids, which will then help to alleviate the herbivore pressure on the plant.

A positive effect on the herbivores could be mediated by a mycorrhiza-induced increase in plant size or amendment (quantity and/or quality) of plant nutrients, making the plant a more favorable place for feeding, development and reproduction (Koricheva *et al.* 2009). Hoffmann *et al.* (2009), for example, showed that adult females of *T. urticae* preferentially feed and oviposit on bean plants that are colonized by the AMF *G.*

mosseae. This symbiosis caused a shortening of the developmental times of the spider mite eggs as well as an increase in the oviposition rates and the female offspring proportion during peak oviposition. These changes could be especially attributed to an increase in P content of the aboveground tissues of the bean plants, which suggests that mycorrhiza-colonized plants represent higher quality host plants than plants without mycorrhiza.

1.1.3.2. Effects of aboveground herbivores on arbuscular mycorrhiza

In general, aboveground herbivores usually negatively affect mycorrhiza (Gehring & Whitham 2002) often causing a reduction in AMF colonization and changing the soil fungal community composition (Van der Heijden & Sanders 2002, Gehring & Whitham 2002). Both of these effects are conditional and vary depending on the surrounding environmental conditions. In general, the effects of the herbivores on mycorrhiza depend on the type of mycorrhizal fungus, whether it forms an ectomycorrhiza (EM) or an arbuscular mycorrhiza (AM). The consumption of photosynthetic tissue by the herbivores could cause a decline in mycorrhizal colonization of the roots due to a decline in photosynthate production supporting the AM (Gehring & Whitham 2002). Both AM and EM consume large amounts of photosynthates. The C demands range from 10 to 50% for EM and 10 to 20% for AM of total primary production. This difference in C demand could signify a change in the type of mycorrhizal fungi communities, changing from a high C demanding EM to a low C demanding AM, when the plant grows under conditions limiting photosynthate production. The loss of photosynthetic tissue by herbivory could also lead to shifting originally mutualistic species towards parasitic ones. However, in some plants, attacks of aboveground herbivores may cause an increase in root biomass and root exudation, causing an increase in mycorrhiza colonization level (Gehring & Whitham 2002).

1.2. Plant volatiles and their role

1.2.1. Plant volatile emissions and herbivores

Volatiles emitted by plants may be constitutively present or be induced by external factors. Every plant response induced by an attack of an herbivore is starting from a constitutive level of emitted volatiles with variation among individual plants (Dicke 2000), even sometimes being at very low concentration or undetectable before damage

is inflicted (Scutareanu *et al.* 2003). Plants have the ability to synthesize and emit a large variety of volatile organic compounds. Some volatiles are common for all plants, while others are specific to one or a few related species of plants (Pichersky & Gershenzon 2002). More than 1000 organic compounds have been reported to be emitted from plants. The substances reported are largely lipophilic products with molecular masses under 300 g/mol and most of them can be assigned to the following classes (in order of decreasing size) (Dudareva *et al.* 2004):

- a) Terpenoids
- b) Fatty acid derivatives
- c) Benzenoids & phenylpropanoids
- d) C₅-branched compounds
- e) Various N and S containing compounds

The majority of these products are emitted from vegetative parts as well as from the flowers and some from the roots. In scented species the flowers produce the highest quantity and the most diverse volatiles. The volatile emission increases during early stages of organ development and, in some species, the emission from flowers and leaves shows a great variation throughout the photoperiod (Dudareva *et al.* 2004). It has been found that some volatile compounds are synthesized *de novo* in the tissues from which they are emitted. Their biosynthesis normally occurs in the epidermal cells of plant tissues from which they can easily escape into the atmosphere or rhizosphere after their synthesis, or in some cases, like in the case of peppermint (*Artemisia annua*) and sweet basil (*Ocimum basilicum*), in the secretory structures of glandular trichomes. The emission of volatiles, and the yield and composition of essential oils are greatly influenced by environmental factors such as light, temperature and relative ambient humidity status (Dudareva *et al.* 2004). For example, temperature increases the emission rates of VOCs exponentially up to an optimum by enhancing the synthesis through enzymatic activities, raising the VOC vapour pressure and decreasing the resistance of emission pathways (Peñuelas & Llusà 2001). Apart from biotic factors, also physical disturbance by wind, rain, hail, or harvesting may affect the production of VOCs in tissues close to the wounding site. Rupture of storing structures allows a direct volatilization of stored VOCs leading to a long-lasting increased emission. Wind may facilitate emission by decreasing diffusive resistance (Peñuelas & Llusà 2001).

Plant-emitted chemical compounds have a wide diversity of purposes and one example is the attraction of pollinators through compounds emitted by the flowers, which can simulate the odors of either receptive or non-receptive bees (*Andrena nigroaenea*). In contrast, other volatiles such as isoprene have the function to aid the plant in some physiological processes. On some tree species, isoprene is believed to increase the general thermal tolerance of photosynthesis (Pichersky & Gershenzon 2002).

Many plant-inhabiting herbivorous and carnivorous arthropods use volatile chemicals emitted by the plant in search for food mainly before but also after physical contact with the plant (Dicke & Van Loon 2000). Many of these secondary chemicals are exploited during selection by specialist herbivores that are not negatively affected by the plant chemicals and may also be exploited by specialist herbivores through sequestration resulting in the protection from their enemies (Dicke 2000). These volatile chemicals may be constitutively present or induced by herbivore attack, with the latter providing reliable information of prey or host presence for foraging carnivores (Dicke & Van Loon 2000). Host plant selection by herbivores may also be affected by the presence of competitors and natural enemies on the plant (Dicke 2000). For spider mites, it has been shown that clean lima bean plants (*Phaseolus lunatus*) emit volatiles that attract *T. urticae* (Dicke 1986). When the spider mites and other herbivores start feeding on the plant they induce the production and emission of volatiles commonly attracting the natural enemies of the herbivores, which is thus considered an indirect defense response of the plant (Dicke & Van Loon 2000). These induced volatiles may also be perceived and used by other herbivores in the surrounding and are thus relevant for this study. The herbivore-induced volatiles may mediate both direct and indirect defenses, and even signal to nearby plants (Pichersky & Gershenzon 2002). The changes in volatiles after herbivore attack can be quantitative or qualitative. The emissions are usually not limited to the site of attack but the volatiles are systemically released. In some tritrophic systems there is high specificity in blend composition, referring to consistent differences in volatile blends of plants that have been damaged by different herbivore species and/or in discrimination by carnivores (Dicke & van Loon 2000).

1.2.2. Effects of arbuscular mycorrhiza on plant volatile emission

Despite their evident physical separation, below- and above-ground organisms associated with plants have the capacity to influence each other via the plant. One type

of these below-aboveground interactions would be if belowground organisms change the chemical composition of aboveground emitted plant volatiles used by foraging herbivores or carnivores (Bezemer & van Dam 2005).

It has been recently shown that AM may cause changes in constitutive and herbivore-induced plant volatiles (HIPVs). For example, aphid parasitoids were more attracted to volatile blends released by plants inoculated with AMF than to those released by plants without AMF (e.g. Bezemer & van Dam 2005). Related to the study system worked with in this thesis, Schausberger *et al.* (2012) showed that AM quantitatively and qualitatively changed the emission of constitutive volatiles and HIPVs of common bean plants (*P. vulgaris*), causing a stronger attraction of the predatory mite *Phytoseiulus persimilis* to the HIPVs of mycorrhizal plants than to those of non-mycorrhizal plants.

1.3. Objectives & hypotheses

It is known that the symbiosis between AMF and plants is in most cases beneficial for both organisms; hence it is considered mutualistic (Read 1998). The mycorrhizal symbiosis can enhance the plant's ability to acquire nutrients from the soil, particularly P and N (Read 1998). These improvements can cause a decrease or an increase in herbivore performance mainly due to the fact that the plant stands either as a better food source for the herbivores or enhances the plant's defensive system (e.g. Koricheva *et al.* 2009). For the two-spotted spider mite *T. urticae*, it has been shown by Hoffmann *et al.* (2009), that the mites achieve a higher fitness and population growth rate on bean plants inoculated with AMF than on non-mycorrhizal bean plants. Since the mycorrhizal symbiosis causes changes in the chemical composition of the plant, it may also change the volatiles emitted by the plants. Indeed, it has been shown that these mycorrhiza-induced alterations in volatile compounds cause a higher attraction of predatory mites *P. persimilis*, which are natural enemies of the two-spotted spider mite *T. urticae*, to mycorrhizal than non-mycorrhizal bean plants (Schausberger *et al.* 2012).

However, it is unknown whether these mycorrhiza-induced changes in plant volatiles also result in a higher attraction of the herbivore itself. Based on the previous findings that the spider mites perform better on mycorrhizal bean plants (Hoffmann *et al.* 2009), mycorrhiza changes the volatiles of bean plants (Schausberger *et al.* 2012) and the foraging spider mites orient themselves to plant volatiles (e.g. Dicke 1986), we

hypothesized that the spider mites *T. urticae* will be more strongly attracted to mycorrhiza inoculated plants than to non-inoculated plants. Furthermore, we also hypothesized that experience plays an important role in host plant selection. Learning by spider mites in the context of host plant choice has been documented before (e.g. Egas & Sabelis 2001) and is called adaptive learning if the mites learn to select the host plant which signifies a greater fitness benefit for them (Papaj & Lewis 1993). We predicted that exposing the mites to the volatiles of mycorrhizal plants coupled with feeding experience for a certain period of time will influence their behavior in host plant selection, resulting in a preference for the volatiles of mycorrhizal plants.

1.4. Experiments

To test the aforementioned hypotheses we performed two Y-tube olfactometer experiments.

- 1) Testing the choice of both mycorrhiza-naive and mycorrhiza-experienced spider mites to odors from clean non-mycorrhizal plants (-M) and clean mycorrhizal plants (+M).
- 2) Testing the choice of mycorrhiza-naive and mycorrhiza-experienced spider mites to odors from spider mite-infested non-mycorrhizal plants (-M/SM) and spider mite-infested mycorrhizal plants (+M/SM).

2. MATERIALS & METHODS

The establishment of the mycorrhizal and non-mycorrhizal plants used in the study was done following the protocol described by Hoffmann *et al.* (2009).

2.1. Plants and mites

2.1.1. The plants: *Phaseolus vulgaris* L.

Leaflets used for the olfactometer choice tests came from common bean plants (*P. vulgaris* var. Taylor's Horticultural) grown under controlled environmental conditions [$60 \pm 5\%$ RH, 16:8 h (light:dark period) L:D, 23°/18° C L:D].

To generate mycorrhizal and non-mycorrhizal bean plants, surface-sterilized (75% commercial bleach for 5 min, rinsed with distilled water) seeds of *P. vulgaris* were pregerminated in perlite, which was previously autoclaved for 20 min at 121 °C. After 8 to 10 days, two seedlings per pot were transferred into 1 liter pots and grown in a 1:1:1 silicate sand/expanded clay/soil substrate mixture, which was previously autoclaved for 20 min at 121 °C (Hoffmann *et al.* 2009).

The previously described process was performed for both experiments. In the first experiment, we only used clean non-infested bean plants. In the second experiment, the establishment of spider mite-infested plants was done by adding 30 adult spider mite females to each plant, randomly distributed throughout the plant using a fine slightly moistened camel's hair brush. On the fifth day after starting the infestation, before hatching of the eggs laid by the spider mite females occurred, the plants were used in olfactometer tests. Using the plants before hatching of the spider mite eggs was necessary to keep the densities and thus the damage caused by the spider mites similar between mycorrhizal and non-mycorrhizal plants.

2.1.1.1. Mycorrhizal & non-mycorrhizal plants

To create the mycorrhizal plants we added ~5 g per plant of *Glomus mosseae* inoculum (BEG 12; International Bank of Glomeromycota <http://www.kent.ac.uk/bio/beg>) into each of the planting holes. The substrate containing the mycorrhiza inoculum was repeatedly used to grow new plants to guarantee the presence of the mycorrhizal fungus and to accelerate the time of root colonization by the fungus. For non-mycorrhizal plants a water filtrate of the inoculum was added to each of the pots. This was done to

provide the same conditions for both mycorrhizal and non-mycorrhizal plants with the only difference being the presence or absence of the mycorrhizal fungi.

Once the plants were planted in the new pots, they were left to grow for 2 to 4 weeks under standardized environmental conditions ($60\pm 5\%$ RH, 16:8 h L:D, 23/18°C L:D). The plants were watered once a week with a P-reduced nutrient solution ($\text{Ca}(\text{NO}_3)_2$ 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, $\text{Fe}_6\text{H}_5\text{O}_7 \times 3\text{H}_2\text{O}$ 50 mg/l, $\text{Na}_2\text{B}_4\text{O}_7 \times 4\text{H}_2\text{O}$ 1.3 mg/l, $\text{MnSO}_4 \times 4\text{H}_2\text{O}$ 1.5 mg/l, $\text{ZnSO}_4 \times 7\text{H}_2\text{O}$ 0.6 mg/l, $\text{CuSO}_4 \times 5\text{H}_2\text{O}$ 0.54 mg/l, $\text{Al}_2(\text{SO}_4)_3$ 0.028 mg/l, $\text{NiSO}_4 \times 7\text{H}_2\text{O}$ 0.028 mg/l, $\text{Co}(\text{NO}_3)_2 \times 6\text{H}_2\text{O}$ 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 two times per week with a NO_3^- fertilizer (KNO_3 1.01g/l, CaNO_3 2.36 g/l) to avoid nodulation of rhizobia.

2.1.1.2. Mycorrhizal colonization level analysis

The roots of all plants used in experiments were checked for mycorrhizal colonization allowing us to assign a plant-specific level of root colonization by AMF to each of the leaflets used in the olfactometer tests. After the olfactometer tests, plants were removed from the pots exposing the roots, the remaining soil sticking to the roots was rinsed off with cold tap water. A portion of the roots of around 2 to 3 cm was cut immediately after rinsing, taking care to leave a considerable amount of intact roots for possible further tests. Root samples were first boiled in 10% KOH for 10 min at 90 °C in order to clean them, after boiling with KOH the remaining liquid was removed and then stained by boiling them for 7 to 9 min at 90 °C in a 5% ink (Schaeffer black ink) house-hold vinegar (equal to 5% acetic acid) solution. After staining the roots were again rinsed with cold tap water (Vierheilig *et al.* 1998) (Fig 1).

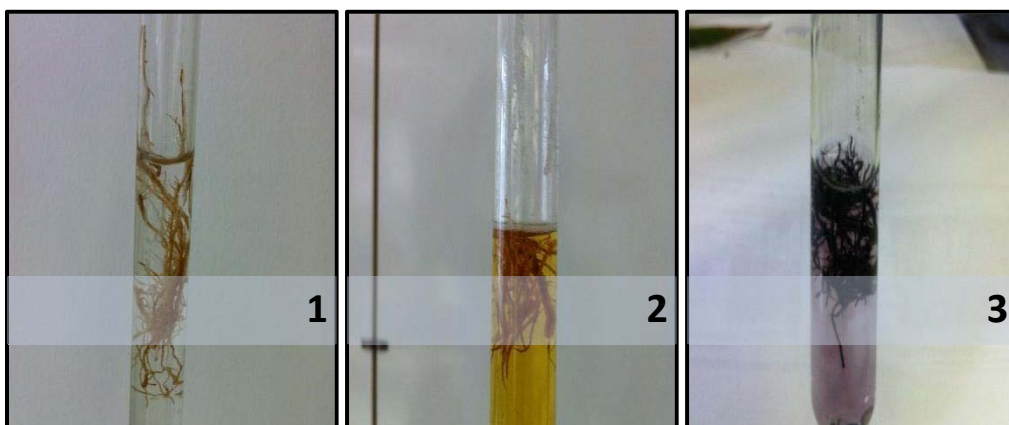


Fig. 1: (1) fresh root samples; (2) roots after boiling with 10% KOH; (3) roots after staining.

The estimation of the percentage of root length colonized by the AMF (RLC) was estimated according to Newman (1966), using a modified gridline intersect method (Giovannetti & Mosse 1980) (Fig 2).

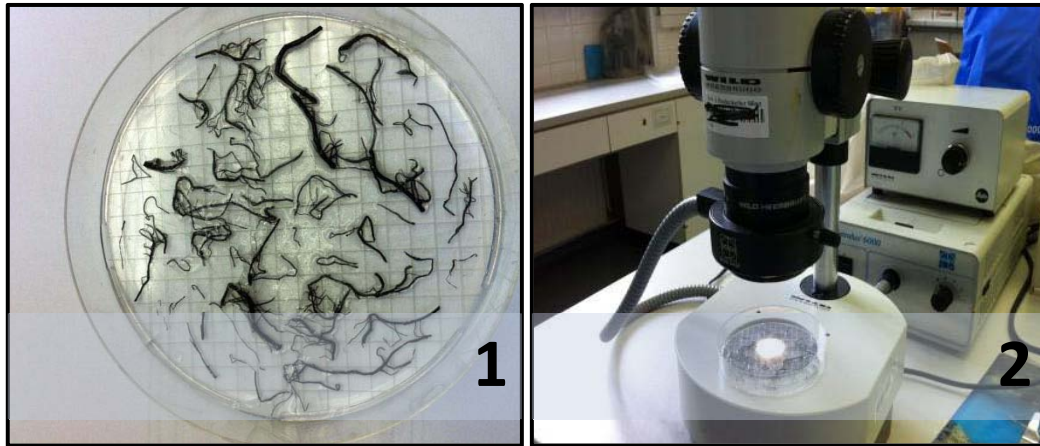


Fig. 2: (1) stained root sample in a petri dish ready for the counting of root length colonized by AMF; (2) counting of AMF colonized roots with the help of a dissection microscope.

2.1.2. The mites: *Tetranychus urticae* Koch

The population of two-spotted spider mite *T. urticae* used for this study was maintained on whole non-mycorrhizal common bean plants *P. vulgaris*, grown under controlled environmental conditions (25 ± 5 °C, 60-80% RH, 16:8 h L:D).

In the olfactometer tests we used mycorrhiza-naive and -experienced adult spider mite females. Each mite was starved for 16 to 20 h before the olfactometer tests, by placing it in an empty circular acrylic cage (1.5 x 0.3 cm) without any food (Schausberger 1997).

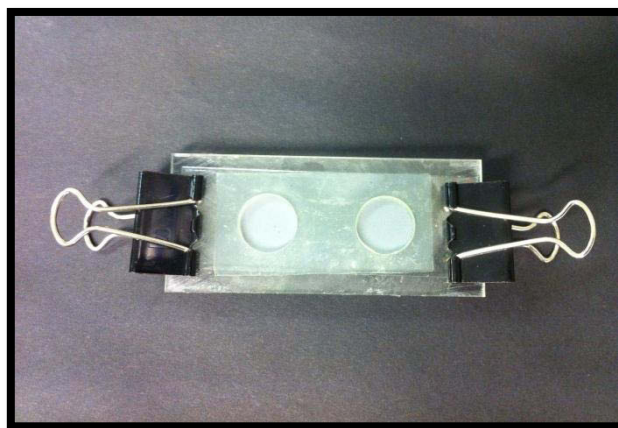


Fig. 3: Acrylic cages used to starve the mites before the olfactometer test (Schausberger 1997).

2.1.2.1. Mycorrhiza-naive mites

Mycorrhiza-naive mites were reared on non-mycorrhizal bean plants (*P. vulgaris*) for 6 to 10 days before the olfactometer experiment took place. Around 30 to 40 female adult mites were placed randomly on each plant using a slightly moistened fine camel's hair brush. Plants were kept under controlled environment conditions (25 ± 5 °C, 60-80% RH, 16:8 h L:D).

2.1.2.2. Mycorrhiza-experienced mites

Mycorrhiza-experienced mites were reared on whole bean plants (*P. vulgaris*), previously inoculated with the arbuscular mycorrhizal fungus *G. mosseae*, for 6 to 10 days before the olfactometer experiment took place and kept under controlled environmental conditions (25 ± 5 °C, 60-80% RH, 16:8 h L:D). Around 30 to 40 mites were randomly placed on each mycorrhizal plant.

2.2. Y-tube olfactometer choice tests

2.2.1. Olfactometer

The Y-tube olfactometer used for the choice tests (see Schausberger *et al.* 2012 for details) was a modification of the olfactometer described by Sabelis & van de Baan (1983), consisting of three glass tubes with an equal inner diameter (40 mm) and length (130 mm), which were melted together in a Y-shape (Fig. 4). The two upper arms or choice arms were joined together at 75° at the intersection forming an angle of 142.5° between each one of the choice arms and the base arm. Each of the choice arms was connected to a set of three tubular acrylic chambers. Each chamber had a total length of 55 mm and an inner diameter of 35 mm (Fig. 4). The outer chamber contained activated charcoal in order to purify the incoming air; the middle chamber contained the leaf (odor) sample and was connected to the other two chambers through female joints. The inner chamber was connected to the choice arm through a male joint, reaching approximately 20 mm into the choice arm. The inner and outer chambers were sealed with gauze to avoid that the spider mites could reach the leaf sample and keep the charcoal inside the outer chamber, respectively. A Y-shaped stainless wire was placed inside the Y-shaped glass tube in equidistance to the inner walls of the Y-tube, serving as a walking path for the mites. The wire started 20 mm inside the bottom end of the base arm, where the mites were released, branched at the point of the intersection of the

three arms and reached to the end of both choice arms. The wire was fixed by a perpendicular 20-mm-long extension and held together by an inert plastic piece placed into a small hole on the upper wall of the base arm. Also at the end of each choice arm, the wire was kept in place by introducing the wire through a small hole in the gauze of the inner chamber. During tests, the olfactometer was placed on a table covered by black cardboard and a cold light lamp was centered above the intersection of the Y-tube to avoid any bias in lighting towards one or the other arm. Air was sucked through the Y-tube with a flow rate of $2.5 \text{ liter min}^{-1}$ per arm, totaling $5.0 \text{ liter min}^{-1}$ at the end of the base arm using a mini-diaphragm-vacuum pump (Laboport® N86 KN.18; KNF Neuburger, Freiburg, Germany) connected to the bottom end of the base arm.

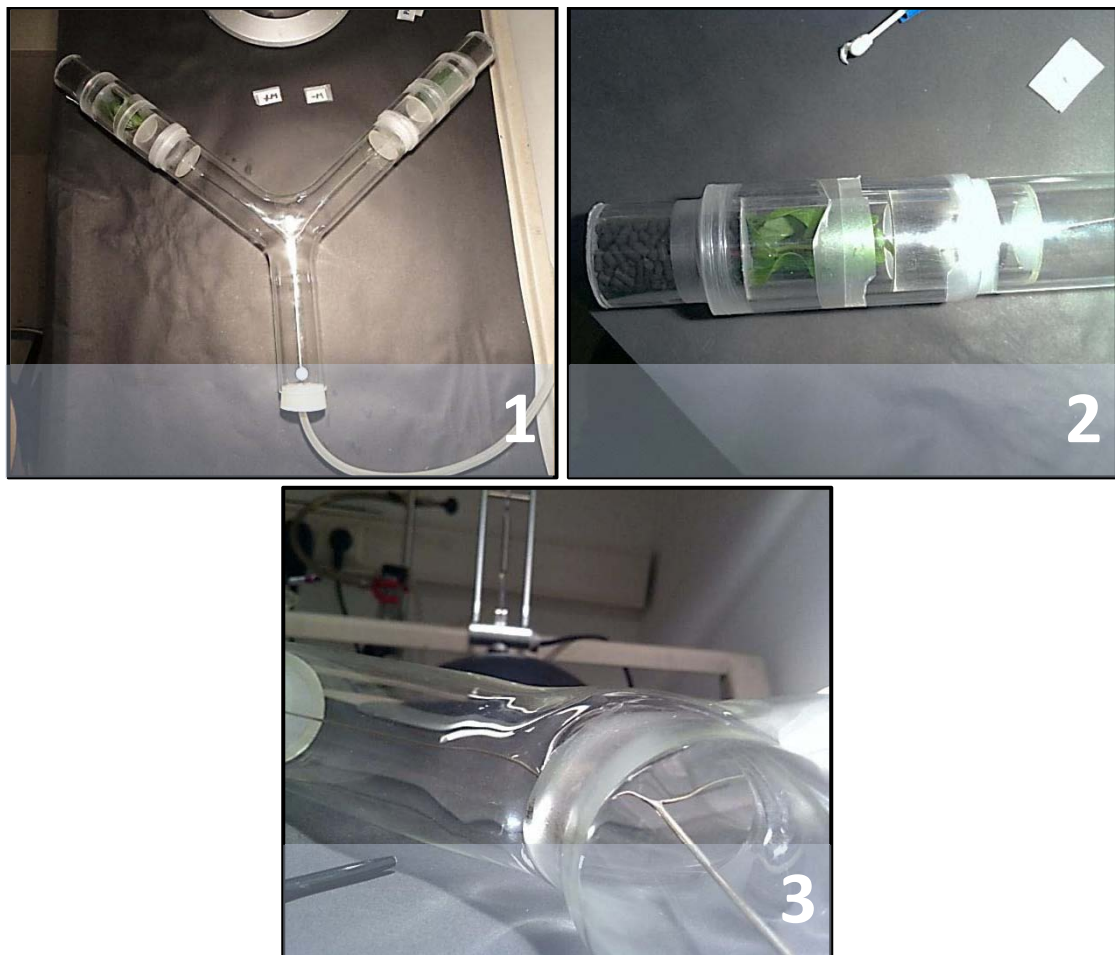


Fig. 4: (1) Y- tube olfactometer set-up (Schausberger *et al.* 2012); (2) set of tubular acrylic chambers; (3) stainless wire running through the base arm and both choice arms.

2.2.2. Choice tests

In the first experiment, each spider mite female was given a choice between two different odor sources, one emanating from clean non-mycorrhizal bean leaflets and the other emanating from mycorrhizal bean leaflets. In the second experiment, the odors came from spider mite-infested non-mycorrhizal and mycorrhizal bean leaflets. Each leaf sample consisted of the youngest fully developed trifoliate leaf, detached from the plant. Detached leaves were used because this allows a better standardization of age, functional part and biomass of the plant material than using whole plants. Moreover, using detached leaves allows excluding any influence of volatiles from other parts such as the substrate or the roots (Schausberger *et al.* 2012).

Each spider mite was individually picked up from the starvation cage using a slightly moistened camel's hair brush and placed on the bottom end of the wire inside the base arm (approximately 2 cm above the bottom end of the base tube). After release, each mite was observed for a maximum time of 5 min. If during this period the mite managed to reach the end of either one of the choice arms, it was considered to be responsive, and the choice arm (left or right), source of odor (+M or -M) and response time were recorded. If during this period the mite did not reach the end of a choice arm, it was judged as being non-responsive. Mites falling down from the wire were not considered for analysis. After every mite tested, an ethanol-moistened cotton swab was used to clean the wire up to slightly after the intersection of the choice arms. After every 5 mites, the wire was taken out from the glass tube and fully cleaned with an ethanol-moistened tissue, and the acrylic chambers were switched to the opposite sides of the olfactometer to avoid any inadvertent bias to one side that could alter the mites' choice. For every sample pair of leaves, 10 mites, 5 mycorrhiza-naïve and 5 mycorrhiza-experienced, were tested. Within each leaf sample pair, the mites were released in an alternated (naïve – experienced – naïve – experienced, etc.) order.

2.3. Statistical analyses

The program SPSS 15.0 (SPSS Inc. Chicago, IL, USA) was used for all statistical analyses. Data of experiment 1 and 2 were analyzed separately. Within each experiment, separate binary logistic regressions were performed to assess the effects of mycorrhization level on responsiveness (yes/no) of mycorrhiza-naïve and mycorrhiza-experienced mites.

In order to assess whether the mycorrhiza-naïve and mycorrhiza-experienced mites had a preference for the odors of clean mycorrhizal plants (+M) or clean non-mycorrhizal plants (-M) in experiment 1, as well as the odors of infested mycorrhizal plants (+M/SM) and infested non-mycorrhizal plants (-M/SM) in experiment 2, two-tailed binomial tests were performed within each group of mites, mycorrhiza-naïve and -experienced (assuming a 0.5 probability to choose the odor of mycorrhizal and non-mycorrhizal leaf samples). Within each experiment, a univariate analysis of variance (ANOVA) was used to analyze whether the response time of the mite females to one of the two odor sources was influenced by the type of odor (from mycorrhizal plants or not) and mycorrhiza-experience.

3. RESULTS

3.1. Root length colonization (RLC)

All plants used for the olfactometer tests and generating the mycorrhiza-experienced mites were analyzed for mycorrhization level after the completion of the olfactometer tests. Only plants that were inoculated with *G. mosseae* showed the presence of AMF in their roots (Table 1). All non-inoculated plants had 0% RLC.

Root Length Colonization (RLC)			
Experiment	# of plants	Average RLC	SD
1	16	19%	7.9
2	19	18%	5.5

Table 1: Average RLC and standard deviation (SD) of mycorrhizal plants used for each of the two experiments: (1) clean bean plants; (2) spider mite-infested bean plants.

3.2. Choice tests

3.2.1. Mycorrhiza-naïve mites on non-infested plants

A total of 39 out of 72 naïve mites exhibited a response within 5 min to either the odor of mycorrhizal (+M) or non-mycorrhizal (-M) bean plants (16 to -M; 23 to +M). A two-tailed binomial test showed that mycorrhiza-naïve mites did not have a preference to either one of the odors of clean mycorrhizal and clean non-mycorrhizal plants (Figure 5).

3.2.2. Mycorrhiza-experienced mites on non-infested plants

A total of 38 out of 75 mycorrhiza-experienced mites exhibited a response to either the odor of mycorrhizal (+M) or non-mycorrhizal (-M) bean plants (12 to -M; 26 to +M). A two-tailed binomial test showed that mycorrhiza-experienced mites were more strongly attracted to the odor of mycorrhizal plants than to that of non-mycorrhizal plants (Figure 5).

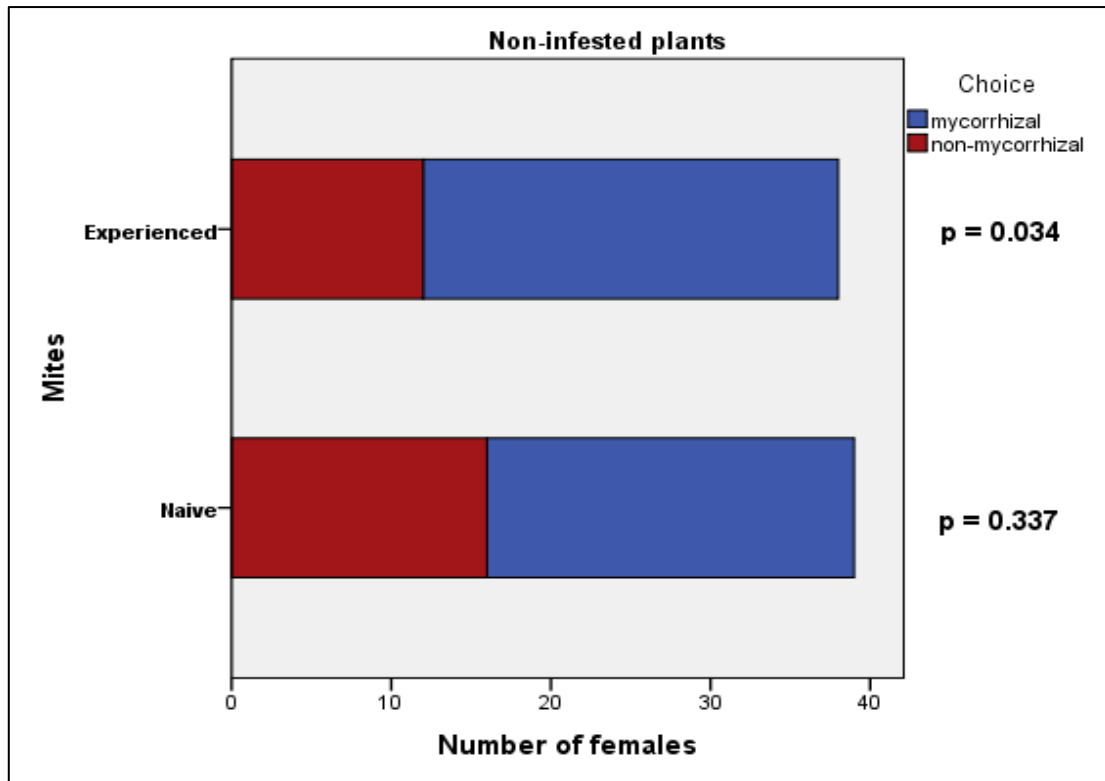


Fig. 5: Number of mycorrhiza-naive and -experienced spider mites having a preference for the odor of clean mycorrhizal or non-mycorrhizal bean plants (*P. vulgaris*) in olfactometer tests. P-values refer to the results of binomial tests assuming random choice.

3.2.3. Mycorrhiza-naive mites on infested plants

A total of 47 mites out of 95 responded to either the odor of mycorrhizal (+M) or non-mycorrhizal (-M) bean plants (23 to -M; 24 to +M). A two-tailed binomial test showed that naive mites did not have a preference for one of the odors (Figure 6).

3.2.4. Mycorrhiza-experienced mites on infested plants

A total of 48 mites out of 95 responded to either the odor of mycorrhizal (+M) or non-mycorrhizal (-M) bean plants (22 to -M; 26 to +M). A two-tailed binomial test revealed that, similar to naïve mites, experienced mites did not have a preference for one of the odors (Figure 6).

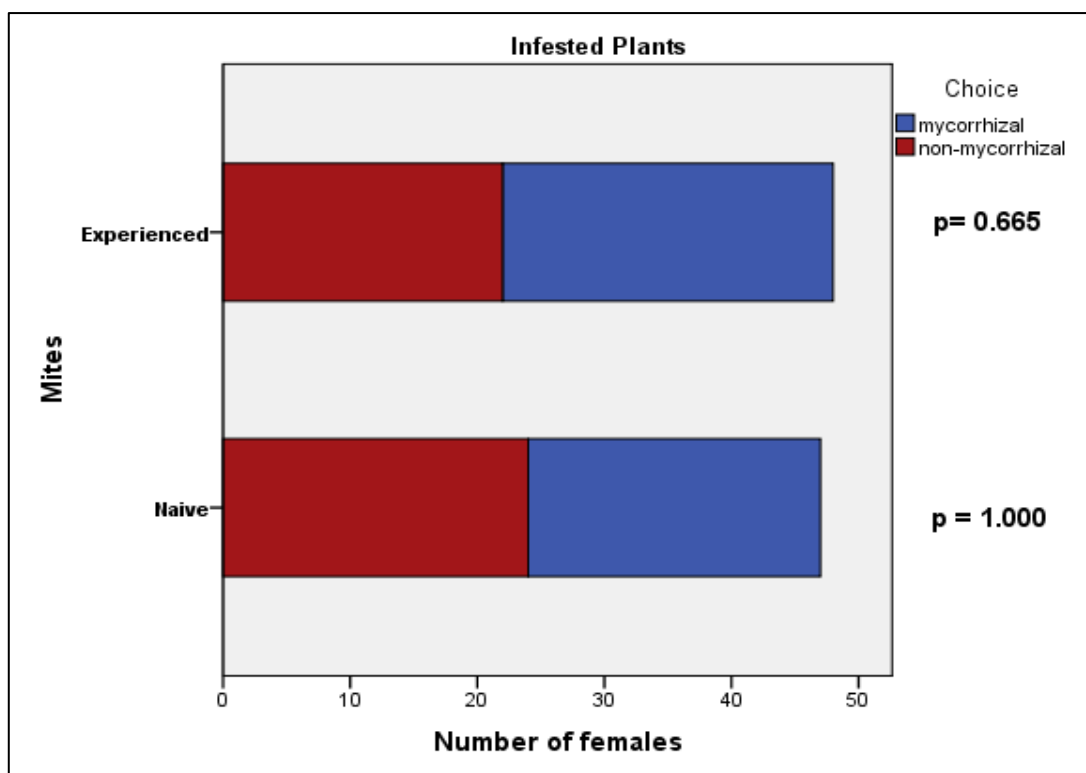


Fig. 6: Number of mycorrhiza-naive and -experienced spider mites having a preference for the odors of spider mite-infested mycorrhizal or non-mycorrhizal bean plants (*P. vulgaris*) in olfactometer tests. P-values refer to the results of binomial tests assuming random choice.

3.3. Response time

3.3.1. Response time towards the odors of non-infested plants

Univariate analysis of variance (ANOVA) revealed that the response time of the mites to either the odor of clean mycorrhizal (+M) or non-mycorrhizal (-M) bean plants was not influenced by mycorrhization (presence/absence) of the plants or by mycorrhiza-experience (Table 2). However, the interaction between mycorrhiza-experience and presence/absence of mycorrhiza indicates that mycorrhiza-experienced mites needed longer to reach the odor source of mycorrhizal plants than that of non-mycorrhizal plants, whereas the response time of naive mites was similar towards either odor source (Figure 7).

Mycorrhiza-naive and -experienced mites on non-infested plants					
Source	Type III Sum of Squares	df	Mean Square	F	P-value
Corrected model	36879.954 ^a	3	12293.318	1.994	0.123
Intercept	1646597.977	1	1646597.977	267.042	0.000
Choice	57.933	1	57.933	0.009	0.923
Mycorrhiza experience	5386.455	1	5386.455	0.874	0.353
Choice*Myc. exp.	22728.192	1	22728.192	3.686	0.059
Error	443956.401	72	5187.084		
Total	2328189	76			
Corrected Total	480836.355	75			

Table 2: Results of ANOVA for the influence of experience and mycorrhization on the response time of mycorrhiza-naive and –experienced spider mites towards the odors of clean mycorrhizal and non-mycorrhizal bean plants (*P. vulgaris*).

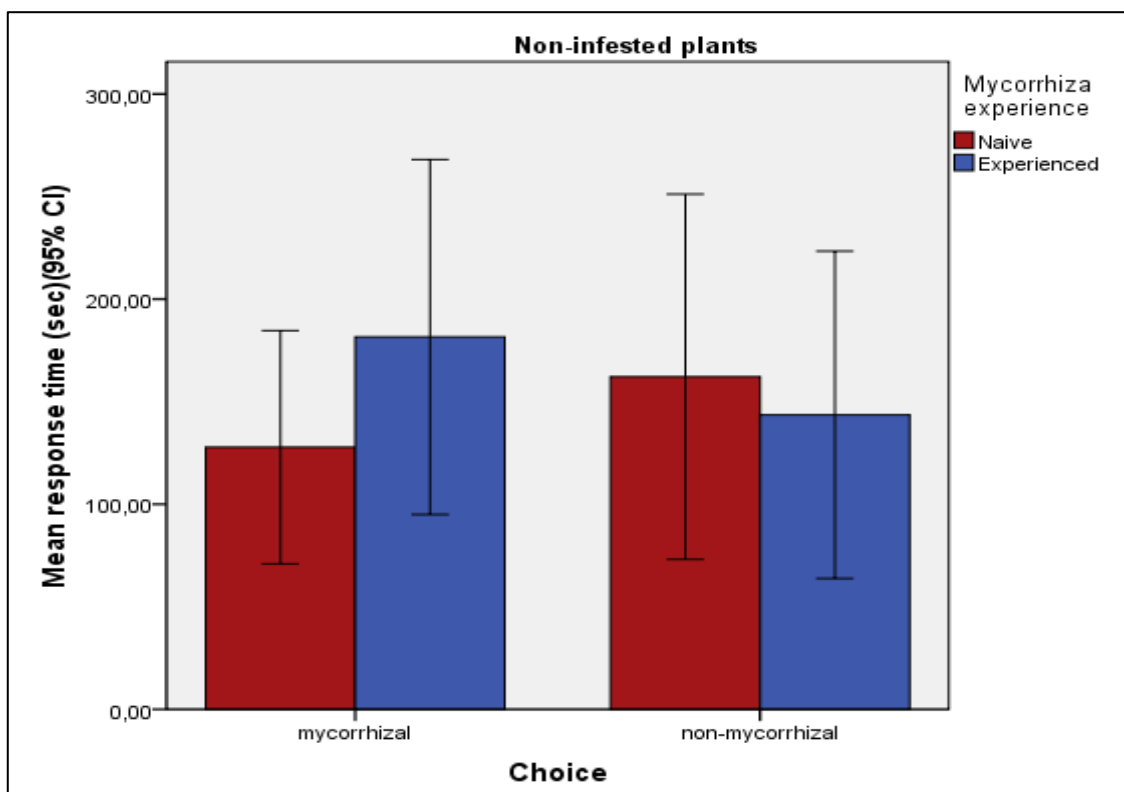


Fig. 7: Mean response time (\pm 95% CI) of both mycorrhiza-naive and -experienced spider mites towards the odors of clean mycorrhizal and non-mycorrhizal bean plants (*P. vulgaris*).

3.3.2. Response time towards the odors of infested bean plants

Univariate analysis of variance (ANOVA) revealed that the response time to either the odor of infested mycorrhizal (+M) or non-mycorrhizal (-M) bean plants was neither influenced by the

presence or absence of mycorrhiza in the plant nor by mycorrhiza-experience (Table 3, Figure 8).

Mycorrhiza-naïve and -experienced mites on infested plants					
Source	Type III Sum of Squares	df	Mean Square	F	P-value
Corrected model	3827.273 ^b	3	1275.758	0.364	0.779
Intercept	2298131.679	1	2298131.679	655.127	0.000
Choice	2591.831	1	2591.831	0.739	0.392
Mycorrhiza experience	1342.851	1	1342.851	0.383	0.538
Choice*Myc. exp.	65.092	1	65.092	0.019	0.892
Error	319220.663	91	3507.919		
Total	2627486	95			
Corrected Total	323047.937	94			

Table 3: Results of ANOVA for the influence of experience and mycorrhization on the response times of mycorrhiza-naïve and -experienced mites towards the odors of spider mite-infested mycorrhizal and non-mycorrhizal bean plants (*P. vulgaris*).

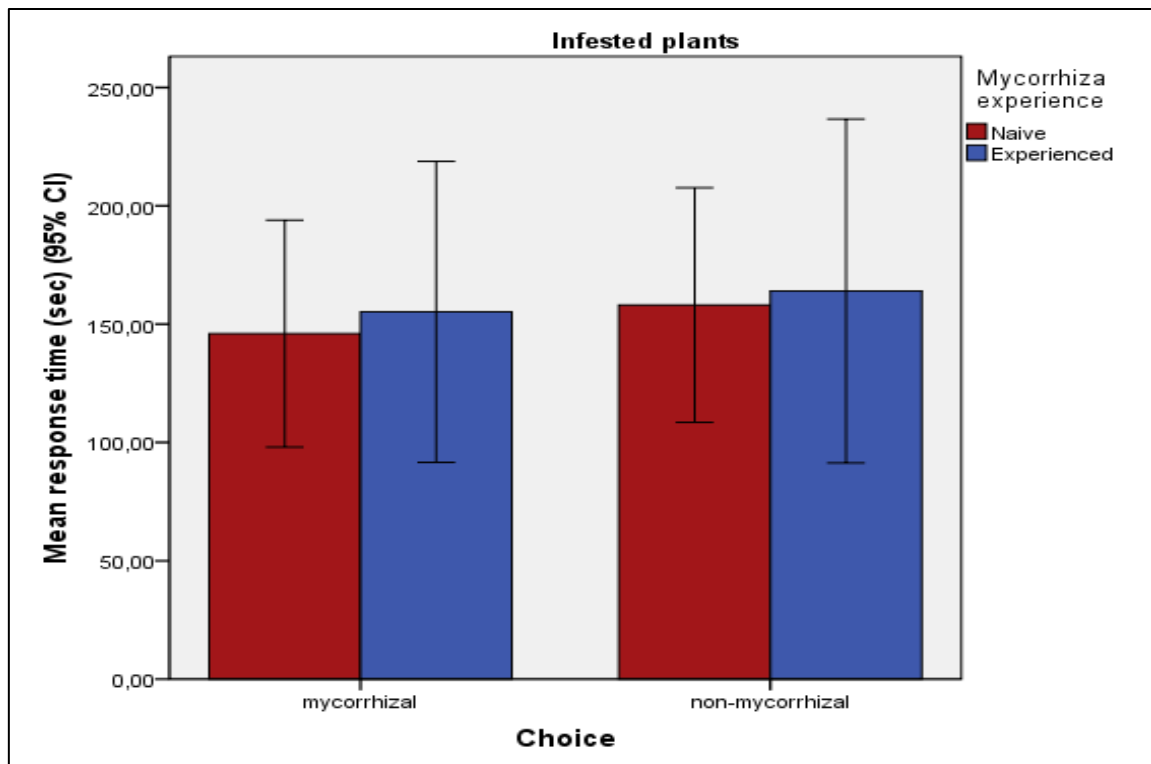


Fig. 8: Mean response time (\pm 95% CI) of both mycorrhiza-naïve and -experienced spider mites to odors of spider mite-infested mycorrhizal and non-mycorrhizal bean plants (*P. vulgaris*).

3.4. Responsiveness influenced by mycorrhization levels

3.4.1. Mycorrhiza-naive and –experienced mites on non-infested plants

Binary logistic regression showed that the responsiveness of naive mites was not significantly influenced by the mycorrhization level of the plants (Table 4, Figure 9). In contrast, responsiveness of mycorrhiza-experienced mites was significantly negatively correlated with the levels of mycorrhization (Table 4, Figure 10).

Responsiveness influenced by mycorrhization level		
Mites	Wald χ^2	P-value (2-tailed)
Naive	1.297	0.255
Experienced	3.937	0.047

Table 4: Results of binary logistic regression on the responsiveness (making a choice within 5 min or not) of spider mites to odors from clean bean plants (*P. vulgaris*) with different levels of mycorrhization by *G. mosseae*.

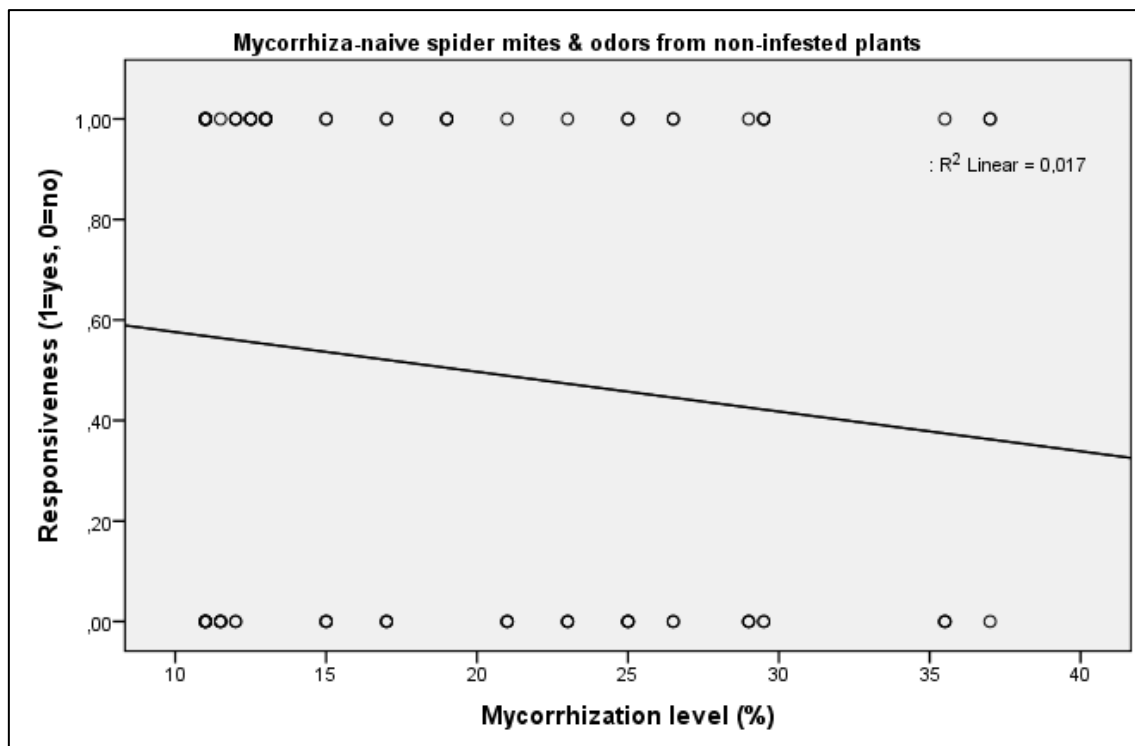


Fig. 9: Correlation of responsiveness (yes/no) of mycorrhiza-naive spider mites *T. urticae* to odors from clean bean plants (*P. vulgaris*) with different levels of mycorrhization by *G. mosseae*.

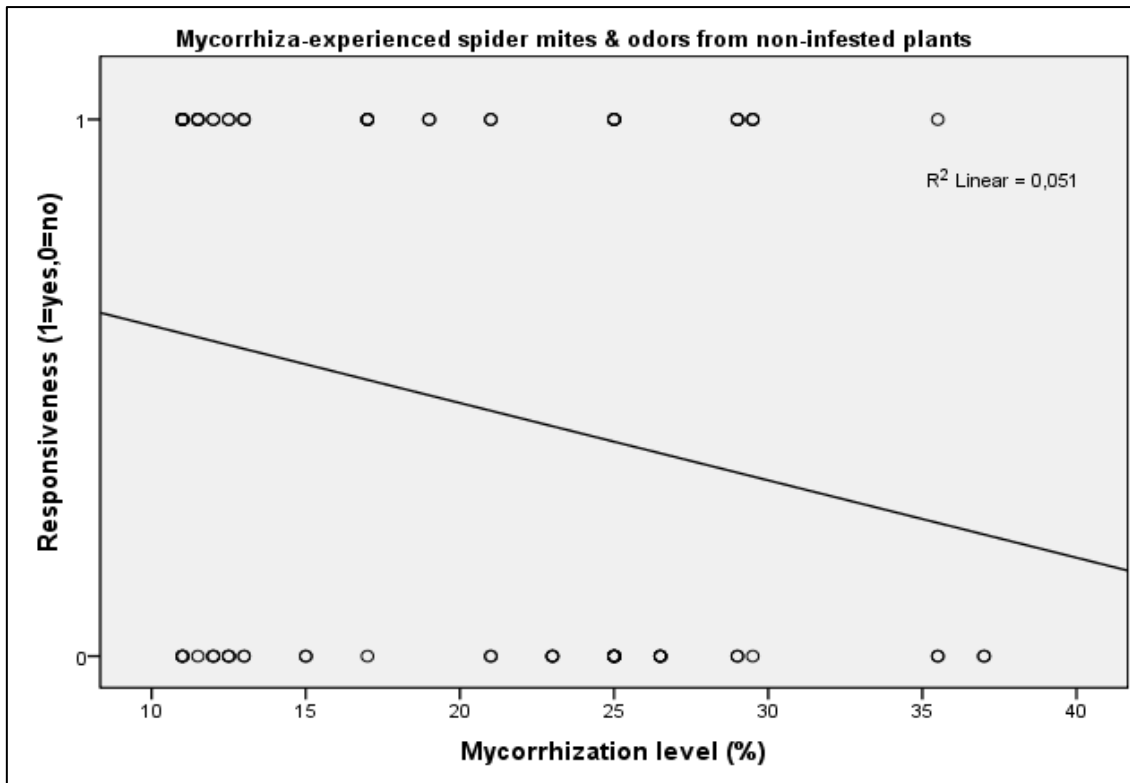


Fig. 10: Correlation of responsiveness (yes/no) of mycorrhiza-experienced spider mites *T. urticae* to odors from clean bean plants (*P. vulgaris*) with different levels of mycorrhization by *G. mosseae*.

3.4.2. Mycorrhiza-naïve and -experienced mites on infested plants

Binary logistic regression showed that neither the responsiveness of mycorrhiza-naïve mites nor that of mycorrhiza-experienced mites was correlated with the level of mycorrhization (Table 5, Figures 11, 12).

Responsiveness influenced by mycorrhization level		
Mites	Wald χ^2	P-value (2-tailed)
Naive	0.479	0.489
Experienced	0.386	0.534

Table 5: Results of binary logistic regression on the responsiveness (making a choice within 5 min or not) of spider mites to odors from spider mite-infested bean plants (*P. vulgaris*) with different levels of mycorrhization by *G. mosseae*.

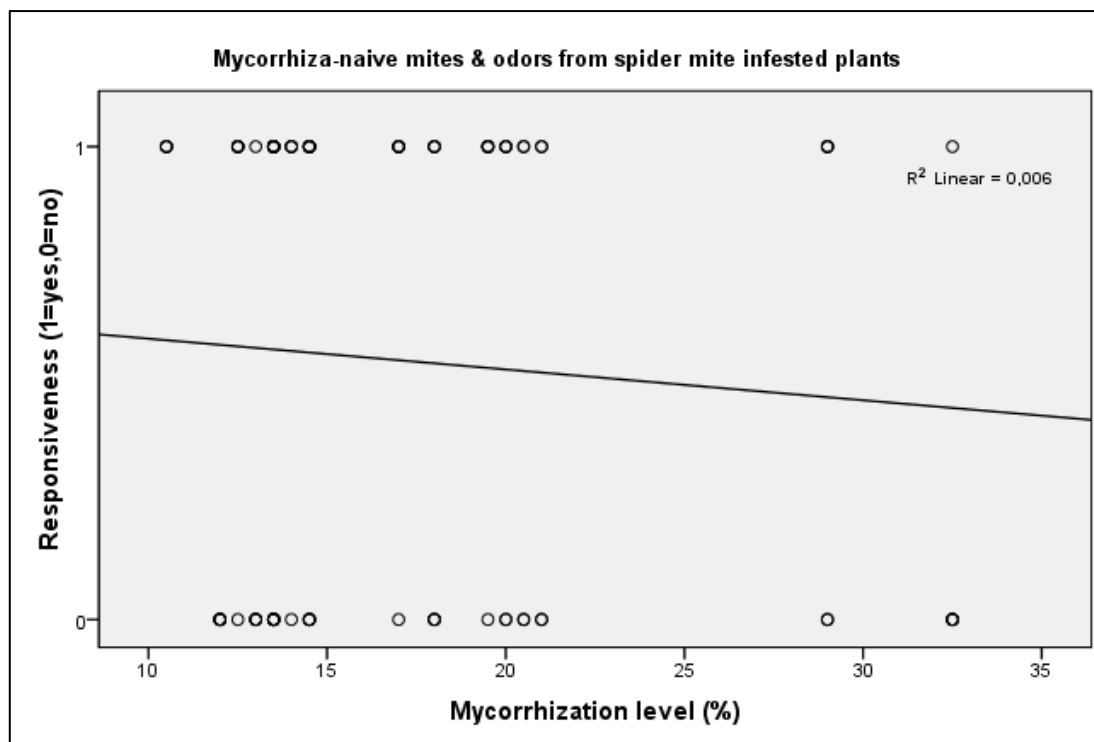


Fig. 11: Correlation of responsiveness (yes/no) of mycorrhiza-naïve spider mites *T. urticae* to odors from spider mite-infested bean plants (*P. vulgaris*) with different levels of mycorrhization by *G. mosseae*.

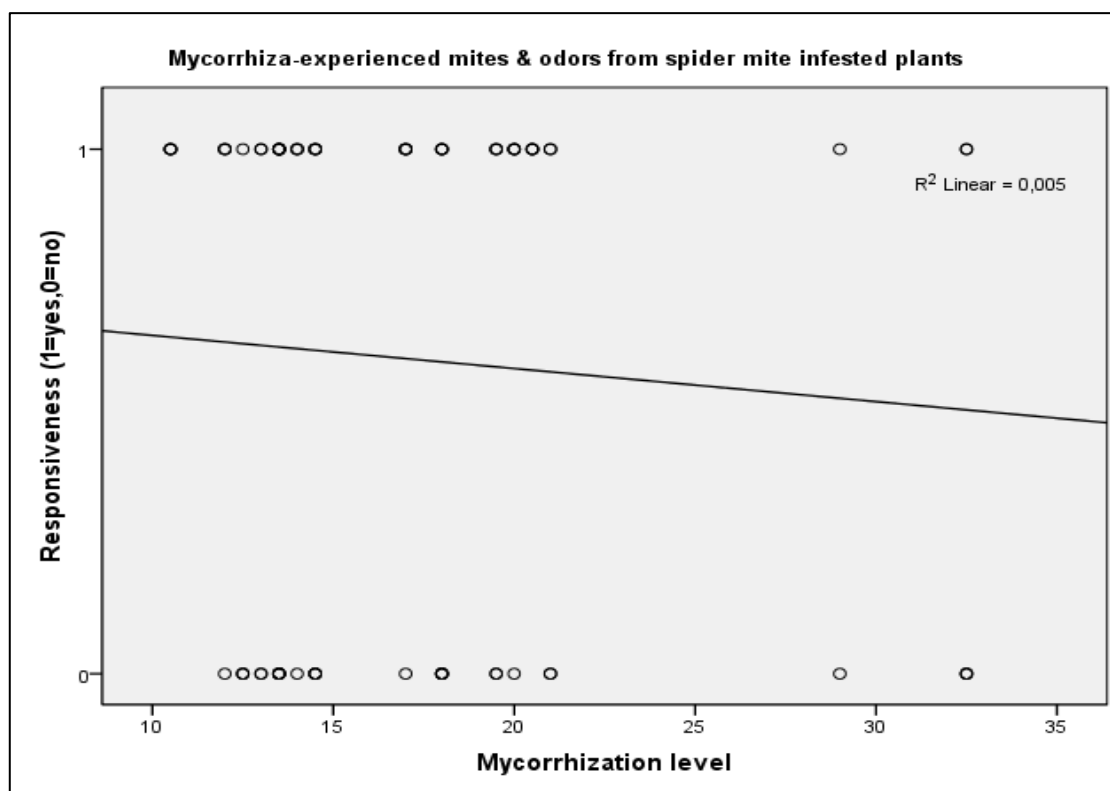


Fig. 12: Correlation of responsiveness (yes/no) of mycorrhiza-experienced spider mites *T. urticae* to odors from spider mite-infested bean plants (*P. vulgaris*) with different levels of mycorrhization by *G. mosseae*.

4. DISCUSSION

Mycorrhizal fungi are ubiquitous soil organisms creating a symbiosis with the roots of about 80% of all plant species. In most cases this symbiosis has positive effects on both interacting organisms, the host plant and the mycorrhizal fungus, and is thus to be considered a mutualistic relation (Smith & Read 2008). There are thousands of studies in this field, most of which deal with the relation between the plant and the fungus. However, mycorrhiza may directly or indirectly also affect other plant-associated organisms such as herbivores feeding on green plant parts (Hoffmann *et al.* 2009, Koricheva *et al.* 2009), root feeding insects (Gange 2001), predators (Hoffmann *et al.* 2011, Schausberger *et al.* 2012), parasitoids (Guerrieri *et al.* 2004) or even pollinators (Wolfe *et al.* 2005). Investigations on the plant-mediated interactions between mycorrhiza and other plant-associated organisms are no simple tasks, mainly because multi-trophic level interactions are highly variable, depending on intrinsic and extrinsic biotic and abiotic factors (van der Heijden & Sanders 2002).

This thesis tries to shed some light on the complex field of multi-trophic below-aboveground interactions, which are ubiquitous in nature but for large parts poorly understood. By analyzing the influence of the AMF *Glomus mosseae* on the attraction of the two-spotted spider mite *Tetranychus urticae* to odors from mycorrhizal and non-mycorrhizal common bean plants *Phaseolus vulgaris*, this thesis aims to increase the understanding of the interactions between below- and aboveground living organisms and to stimulate further research on this issue.

The performed olfactometer choice tests provide evidence that changes in the volatiles of common bean plants (*P. vulgaris*) caused by the symbiosis with AMF (*G. mosseae*) can positively influence the attraction of the two-spotted spider mite (*T. urticae*) to its host plant. This was true for the first experiment where mycorrhiza-experienced and -naive mites were exposed to odors from clean mycorrhizal and non-mycorrhizal bean plants. In this experiment 1, the mycorrhiza-experienced mites showed a clear preference for the odors of mycorrhizal plants while mycorrhiza-naive mites did not show any significant preference for either of the offered odors.

Spider mites and other plant-inhabiting organisms commonly use various cues and sensory modalities to find a suitable host plant (Dicke & van Loon, 2000), one of the utilized signals being plant volatiles. In this context it is important to mention that AM commonly causes changes in the plant volatile emissions (Fontana *et al.* 2009, Leitner *et al.* 2010, Schausberger *et al.* 2012), which, in the multi-trophic system worked with, signaled the experienced spider mites the presence of a higher quality host plant. It is known that AM commonly improves various plant traits by increasing nutrient uptake, especially P and N (Koricheva *et al.* 2009; Read & Pérez-Moreno 2003; Smith & Read 2008), and allowing an easier access to C in the soil, which under normal conditions would be unavailable for the plant (Kapulnik & Douds 2000). For spider mite-bean plant interactions, Hoffmann *et al.* (2009) showed that AM positively affects host plant choice and life history performance of the spider mites, which also showed a clear preference to feed and oviposit on mycorrhizal plants when given a choice between mycorrhizal and non-mycorrhizal plants. Feeding on mycorrhizal plants resulted in a shortened egg developmental time of the offspring and increased oviposition rate and female offspring proportion, altogether enhancing the mites' population growth rates.

Previous experience with the mycorrhizal plant and its volatiles was a decisive factor for host plant selection. Experience allowed the mites to distinguish between the odors of mycorrhizal and non-mycorrhizal plants and to display a preference for the former. Mites kept on mycorrhizal plants for a couple of days apparently learned during this time to associate the smell of the plant with high quality food, amending their life history traits and providing favorable conditions for their reproductive success (Dicke 1986). Such a phenomenon is called adaptive learning (Papaj & Lewis 1993) and has been previously documented for spider mites by Egas & Sabelis (2001), who allowed the spider mites to experience cucumber and tomato plants. The ones that had experienced cucumber had a preference for cucumber plants when given a choice and also their reproductive output was higher on cucumber than tomato. Similar to our study, the mites did not recognize the quality of the plants without experience but required some time to feed on the plants and then come up with a preference. Our results provide further evidence that the spider mites are able to adaptive learning, and to recognize and prefer the odors of mycorrhiza-inoculated bean plants (*P. vulgaris*).

The enhanced attraction of the spider mites to mycorrhizal plants was only apparent when the volatiles were emitted by clean plants, not infested with spider mites. The mycorrhiza-induced changes in the volatiles emitted by spider mite-infested plants did not influence attraction of the spider mites to their host plants. The spider mites randomly chose any of the two odors. Similarly, Guerrieri *et al.* (2004) observed stronger attraction of a 3rd trophic level natural enemy, the aphid parasitoid *Aphidius ervi* (Haliday), to clean mycorrhizal tomato plants and to plants infested with aphids than to plants without the symbiosis and without the aphids.

An interesting result was the difference between both experiments, the first experiment involving clean plants, not infested with spider mites, and the second experiment involving plants infested with spider mites. In the second experiment, neither mycorrhiza-experienced nor -naive mites showed any particular preference to any odor of the infested plants despite the fact that the mycorrhizal fungus *G. mosseae* changes the volatiles of both clean and spider mite-infested bean plants (Schausberger *et al.* 2012). The HIPVs from mycorrhizal and non-mycorrhizal bean plants differ quantitatively and qualitatively and the volatiles of mycorrhizal bean plants are more attractive to the prime natural enemy of the spider mites, the predatory mite *Phytoseiulus persimilis* (Schausberger *et al.* 2012). In general, herbivore-attacked plants emit volatiles in larger amounts than non-attacked plants (Dicke & van Loon 2000). These HIPVs might send mixed signals to other herbivores such as spider mites searching for a suitable host plant. For example, these signals might indicate that the defense system of the plant releasing them has been activated, causing a deterrence of the spider mites to approach that plant. They could also indicate that the herbivores present on that plant have already overcome the defense system and that the plant is already weakened and more susceptible to attack by herbivores. Furthermore, these signals could indicate the presence of competitors and that fewer resources are available, or indicate a higher risk of predation due to attraction of the predators by the HIPVs (Dicke & van Loon 2000).

Some studies have tried to clarify the specific interaction between plants attacked by spider mites and emitting HIPVs and spider mites searching for new host plants. Pallini *et al.* (1997) showed, for example, that spider mites are slightly more attracted to cucumber plants infested with conspecifics than to clean cucumber plants but are

deterred from plants infested with western flower thrips (*Frankliniella occidentalis* Pergande). Assuming that the spider mites used the odors to locate a suitable food resource, in this case the HIPVs would indicate a more suitable food resource or the presence of an already formed web by conspecifics, providing better protection against predators. The blends of HIPVs vary among different plant species and genotypes but also among herbivore species and type of damage inflicted (artificial or herbivory) (Dicke *et al.* 1998). The volatiles from infested bean plants caused the spider mite *T. urticae* to disperse away from the odor source and it is believed that the terpenoid linalool was one of the causes for this behavior (Dicke 1986). Linalool is produced by spider mite-infested bean but not cucumber plants and may thus partly also explain the discrepancy between the results on bean (Dicke 1986) and cucumber (Pallini *et al.* 1997).

The interpretation of the observed lack of discriminatory behavior in the second experiment with infested plants, even for experienced mites, is not an easy task. A possible explanation is that the attractive attributes of the volatiles of AM plants were overshadowed by the change in volatiles through spider mite feeding, indicating intensified competition for resources or even worse, risk of predation. As repeatedly mentioned, HIPVs of mycorrhizal plants are more attractive to the predators of the spider mites, *P. persimilis*, than are HIPVs of non-mycorrhizal plants (Schausberger *et al.* 2012) and may thus indicate a higher risk of predation to the spider mites.

Regarding the response times to odors from clean bean plants in experiment 1, we observed that mycorrhiza-experienced mites choosing the +M odors responded somewhat more slowly than the ones choosing the –M odors. However, it is worth mentioning that only 5 minutes were allowed for the mites to walk the wire and make a choice. It could be that by allowing the mites more time to make a choice the difference in decision-making time between mites choosing the +M and –M odors would have been even greater. A likely explanation is that information processing and decision-making has costs, one of them being the time needed to make a choice among alternative options (e.g. Dukas 2004, Ydenberg 2010). Mites perceiving a difference between the two odors inside the olfactometer needed to decide which arm of the wire they should walk up to reach the preferred +M odor source. In contrast, assuming that the mites choosing the –M odor were indiscriminative, these indiscriminative mites just

walked up the wire without having to stop and compare the two odors. This conclusion is further supported by the negative correlation between the level of mycorrhization and responsiveness, which was only significant in the case of mycorrhiza-experienced mites in the first experiment with clean, non-infested plants. In accordance with the above mentioned time costs of decision-making, it could be that mites perceiving higher levels of mycorrhization were more prone to compare the two odor sources and thus needed more time to decide which arm to walk up than indiscriminative mites. Nevertheless, more detailed research on this issue needs to be conducted in order to get a clearer idea of these behaviors.

5. SYNTHESIS

AM symbiosis is an important ubiquitous phenomenon (Allen 1996). AM commonly enhances nutrient uptake by the plant, which may be to the benefit or detriment of the fitness of herbivorous insects and mites feeding on aboveground plant parts (Koricheva *et al.* 2009). In addition, AM is known to cause changes in the plant's volatile emission (e.g. Fontana *et al.* 2009; Guerrieri *et al.* 2004; Schausberger *et al.* 2012), which may lead to an improvement in the indirect defense system against herbivores (Schausberger *et al.* 2012). To the best of our knowledge, this thesis is the first study to show that mycorrhiza-induced changes in plant volatiles may also be perceived by foraging herbivores, the two-spotted spider mite *T. urticae*. Moreover, the spider mite *T. urticae* has the capacity to adaptively learn to prefer the best quality host plants, which will improve its life history and fitness (Egas & Sabelis 2001). As reported in this thesis, mycorrhiza-experienced spider mites were more strongly attracted to clean non-infested common bean plants *P. vulgaris* that were in symbiosis with the AMF *G. mosseae* than to plants without AM. However, only those mites that experienced feeding and oviposition on mycorrhizal plants and the associated volatiles before the choice tests had the capacity to distinguish between the volatiles of mycorrhizal and non-mycorrhizal plants. The mycorrhiza-experienced spider mites took more time to find and trace the preferred odors, probably reflecting the costs of decision-making. Since mycorrhizal plants are more favorable host plants for the spider mites than are non-mycorrhizal plants, the observed preference of mycorrhiza-experienced spider mites provides evidence of adaptive learning.

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