

Arbuscular mycorrhizal symbiosis increases host plant acceptance and population growth rates of the two-spotted spider mite *Tetranychus urticae*

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Abstract Most terrestrial plants live in symbiosis with arbuscular mycorrhizal (AM) fungi. Studies on the direct interaction between plants and mycorrhizal fungi are numerous whereas studies on the indirect interaction between such fungi and herbivores feeding on aboveground plant parts are scarce. We studied the impact of AM symbiosis on host plant choice and life history of an acarine surface piercing-sucking herbivore, the polyphagous two-spotted spider mite *Tetranychus urticae*. Experiments were performed on detached leaflets taken from common bean plants (*Phaseolus vulgaris*) colonized or not colonized by the AM fungus *Glomus mosseae*. *T. urticae* females were subjected to choice tests between leaves from mycorrhizal and non-mycorrhizal plants. Juvenile survival and development, adult female survival, oviposition rate and offspring sex ratio were measured in order to estimate the population growth parameters of *T. urticae* on either substrate. Moreover, we analyzed the macro- and micronutrient concentration of the aboveground plant parts. Adult *T. urticae* females preferentially resided and oviposited on mycorrhizal versus non-mycorrhizal leaflets. AM symbiosis significantly decreased embryonic development time and

increased the overall oviposition rate as well as the proportion of female offspring produced during peak oviposition. Altogether, the improved life history parameters resulted in significant changes in net reproductive rate, intrinsic rate of increase, doubling time and finite rate of increase. Aboveground parts of colonized plants showed higher concentrations of P and K whereas Mn and Zn were both found at lower levels. This is the first study documenting the effect of AM symbiosis on the population growth rates of a herbivore, tracking the changes in life history characteristics throughout the life cycle. We discuss the AM-plant-herbivore interaction in relation to plant quality, herbivore feeding type and site and the evolutionary implications in a multi-trophic context.

Keywords Acari · Aboveground–belowground interaction · Multi-trophic · Plant quality

Introduction

Most terrestrial plants are associated with root-colonizing fungi establishing a symbiosis commonly referred to as mycorrhiza, with endotrophic arbuscular mycorrhiza being the most common form (reviewed by Smith and Read 1997). In most cases arbuscular mycorrhizal (AM) symbiosis improves the nutritional status of the host plant and thus, its overall fitness (Read 1998). Studies on the interaction between AM fungi and plants are numerous (reviewed by Allen 1996; Koide and Mosse 2004). In contrast, studies on the tripartite interaction between plants colonized by AM fungi and arthropod herbivores feeding on aboveground plant parts are scarce. Existing studies revealed great variability in the effects of arbuscular mycorrhiza on herbivore performance, with effects ranging from negative to neutral

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to positive (reviewed by Gehring and Whitham 2002; Bennett et al. 2006; Gange 2007). Research on this topic has been performed with various host plant, AM fungi and herbivore species. The peculiarity of each of the interacting organisms lead to differing results, hampering the deduction of a general pattern.

Assuming a mutualistic association between plants and mycorrhizal fungi, Bennett et al. (2006) proposed four mechanisms of how improved plant nutrition could possibly affect aboveground plant–herbivore interactions: increase in biomass, nutritive value, tolerance and/or defense of the plant. So far there is no clear evidence for the first mechanism to occur singly, as host plant choice of herbivores, which is considered to be influenced by plant quantity (Schoonhoven et al. 1998), was not affected by increased biomass due to mycorrhizal colonization (Gange and West 1994; Gange and Nice 1997; Gange et al. 2003). Borowicz (1997) and Goverde et al. (2000) suggested that herbivore performance was improved due to amelioration in diet quality, although the nutritive components responsible have not yet been identified. Regarding the third mechanism, Bennett and Bever (2007) and Kula et al. (2005) suggested an effect of AM on plant tolerance to herbivore attack by enhancing growth rates and the ability to compensate for lost photosynthetic surface area. Several studies on AM fungus–plant–herbivore interactions determined a negative effect of AM on the herbivore (e.g., Rabin and Pacovsky 1985; Gange and Nice 1997) but only Gange and West (1994) investigated and found alterations in defense-related secondary plant compounds. Taken together, the studies on the effects of mycorrhizal colonization on herbivory and their varying outcomes suggest that different mechanisms or combinations of mechanisms are applicable for different experimental systems (Bennett et al. 2006). Furthermore, the mechanisms adopted by plants may be influenced by intrinsic traits of mycorrhizal fungi and herbivores. Different genera and species (Hart and Klironomos 2002) and even isolates of the same mycorrhizal species (Munkvold et al. 2004) may differently influence plant performance. Bennett and Bever (2007), who reported on mycorrhizal species and species mixes, and Wooley and Paine (2007), who reported on species isolates, showed that this varying effect may be passed on to the herbivores. Gehring and Whitham (2002) proposed the insect herbivore mode of feeding and host plant specificity to possibly influence the quality of AM-induced changes on herbivore performance. In the majority of studies growth was increased in specialist chewing and specialist and generalist sucking insects, whereas it was decreased in generalist chewing insects. This pattern is in accordance with the assumptions that: (1) specialist herbivores are better adapted to their host plant's secondary metabolite defenses (van der Meijden 1996), (2) the impact of altered defense chemistry and/or

nutrient status of host plants varies between feeding guilds (Borowicz 1997; Awmack and Leather 2002).

We examined the effect of the AM fungus *Glomus mosseae* Nicol. and Gerd. on host plant acceptance and life history of the two-spotted spider mite *Tetranychus urticae* Koch feeding on common bean plants, *Phaseolus vulgaris* L. *T. urticae* is a polyphagous agricultural pest with global distribution and major economic importance (e.g., Helle and Sabelis 1985; Bolland et al. 1998). The mites feed on the plant surface by piercing the parenchyma cells with their stylets and sucking out the cell contents (Tomczyk and Kropczynska 1985). In particular, we determined the ability of *T. urticae* to perceive AM-induced changes in the host plant by subjecting the mites to choice tests between leaves of AM-colonized and non-colonized plants. Furthermore, we quantified AM-induced changes on juvenile survival and development, adult female survival and oviposition and offspring sex ratio of *T. urticae*, and estimated the population growth parameters net reproductive rate (R_0), intrinsic rate of increase (r_m), mean generation time (T), doubling time (Dt) and the finite rate of increase (λ). To the best of our knowledge this is the first study tracking the effects of AM symbiosis on the performance of a herbivore throughout its life cycle.

Materials and methods

Rearing of plants and mites; plant inoculation

Preliminary tests showed a considerable delay from inoculating bean plants until satisfactory root colonization by the AM fungus. To avoid extended plant growth periods in between experiments, a system of constantly active growing units was developed. Surface-sterilized (75% commercial bleach for 5 min, rinsed with distilled water) seeds of *P. vulgaris* var. Taylor's Horticultural were pregerminated in perlite (previously autoclaved for 20 min at 121°C). After 10–12 days the plants were transferred to 1,000-ml pots and five plants per pot grown in a 1:1:1 silicate sand:expanded clay:soil substrate mixture (previously autoclaved for 20 min at 121°C). For mycorrhizal growth pots, ~5 mg per plant of *G. mosseae* inoculum (BEG 12; International Bank of Glomeromycota; <http://www.kent.ac.uk/bio/beg>) was added to the planting holes. For non-mycorrhizal pots, a water filtrate of the inoculum was added. Approximately 2 months after inoculation, pots were considered to contain sufficiently high amounts of active hyphae and all but one plant was cut down. Two pregerminated bean plants obtained according to the procedure described above were added to each pot. As plants and roots were removed for experiments there was a constant change of plants and parts of the substrate in pots. Care was

taken to ensure that at least one, and a maximum of three, living bean plants were always present per pot to guarantee the constant presence of active AM hyphae. Plants used for experiments were left to grow within growth pots for 2–4 weeks under standardized environmental conditions [$60 \pm 5\%$ relative humidity (RH), 16:8 h light:dark (L:D), 23/18°C L:D]. Plants were watered once a week with a P-reduced nutrient solution ($\text{Ca}(\text{NO}_3)_2$ 0.472 g/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 twice a week with a NO_3^- fertilizer (KNO_3 1.01 g/l, CaNO_3 2.36 g/l) to avoid rhizobium nodulation. All plants were checked for mycorrhizal colonization, which allowed us to assign a specific fungal colonization level to each leaf used in experiments. After leaves had been cut off a plant to be used in experiments, the remaining aboveground plant parts and the roots were removed from the pot. The remaining substrate was rinsed off the roots with cold tap water. Roots were cleared by boiling them for 10 min in 10% KOH and stained by boiling for 5 min in a 5% ink (Schaeffer black ink) household vinegar (equal to 5% acetic acid) solution (Vierheilig et al. 1998). The percentage of root length colonized by AM fungus (RLC) was estimated according to Newman (1966). Non-mycorrhizal plants (–M) had 0% RLC. All mycorrhizal plants (+M) had >10% RLC. The average RLC for plants used in herbivore experiments was 38.8 ± 0.67 (SE) %. The stock population of two-spotted spider mites was reared on whole non-mycorrhizal bean plants under standardized environmental conditions ($25 \pm 5^\circ\text{C}$, 60–80% RH, 16:8 h L:D).

Experimental procedures

Choice tests

Each experimental unit consisted of one +M and one –M leaflet, taken from trifoliates, connected by a ~6-cm-long wax bridge (Vanas et al. 2006; Walzer et al. 2006). The leaflets were put upside down with leaflet bases facing each other on moist tissue paper covering a wet sponge ($160 \times 80 \times 80$ mm) placed in a plastic box ($200 \times 100 \times 100$ mm) half filled with water. The edges of the leaflets were covered with moist tissue paper to prevent mite escaping. Single gravid females were randomly taken from the mass-rearing, starved for 16 h and then released in the middle of the wax bridge (one female per experimental unit). Position of and eggs laid by the female on either leaflet were recorded immediately after release and then

after 2, 4, 6, 22, 26, 30, 46, 50, 54, 70, 74 and 78 h. Twenty-nine females were tested and each experimental unit and each female was used only once. Binary logistic regression was used to determine the preference of the female to reside and oviposit on the +M and –M leaflet over time (assuming a 0.5 distribution of female positions and number of eggs). Residence and oviposition site preference (higher number of eggs laid indicating preference) were analyzed for each observation point using binomial tests. Total numbers of eggs laid on either side were compared by Student's *t*-test for paired samples. Unless otherwise stated, SPSS 15.0 (SPSS 2006) was used for all statistical analysis.

Juvenile survival and development

To determine juvenile survival and developmental time of *T. urticae* feeding on +M and –M leaflets, gravid females were randomly taken from the mass-rearing and reared on either a +M or a –M leaf for 72 h to include possible maternal effects. After the 72-h period the females were singly transferred to the experimental arenas. Arenas consisted of square areas (10×10 mm) on trifoliolate –M or +M leaflets, put upside down on moist tissue paper covering a wet sponge ($80 \times 80 \times 80$ mm) placed in a plastic box ($100 \times 100 \times 100$ mm) half filled with water. The arenas were delimited by moist tissue paper to prevent mite escaping. After 6 h the female and all but one egg were removed. Subsequently, survival and development of the spider mites were observed twice per day at 8- and 16-h intervals until the mites reached adulthood or died. Treatments were replicated 23 (–M) and 22 (+M) times, respectively. Developmental progress was determined by the shed skin of the previous life stage. Two-way ANOVA was used to determine differences in developmental times for each developmental stage and total development between treatments and sex.

Adult survival and oviposition, offspring sex ratio

Experimental arenas were constructed as described above with the only difference being the use of whole leaflets taken from trifoliates. The edges of the leaflets were covered with moist tissue paper leaving an accessible surface of ~8 cm². If not disturbed, *T. urticae* females only need to mate once to reach their optimum life fecundity and only the first mating will be effective (Potter et al. 1976). Thus one virgin female teliochrysalis (i.e., the resting stage prior to reaching adulthood) and one adult male, both randomly taken from the mass-rearing, were transferred to either a –M or +M arena. As soon as the first egg was laid the male was removed from the arena. Subsequently, survival of and number of eggs laid by the female were recorded once per day for 14 days after the female had emerged. Treatments were replicated 30 (–M) and 38 times (+M), respectively.

For determination of the offspring sex ratio, eggs were transferred to separate leaf arenas and left to develop to adulthood. To determine possible changes in offspring sex ratio over time, eggs laid on days 1–3, 4–6, 7–9, and 10–13 of either treatment were grouped together on separate arenas. Oviposition of –M and +M females over time was compared by repeated measures ANOVA and time-dependent offspring sex ratios by χ^2 -tests.

Population growth parameters

Data on juvenile survival and development, adult survival and oviposition and offspring sex ratio were used to calculate the population growth rates. Jackknife estimates of R_0 , r_m , T , Dt , and λ were calculated using the program based on SAS 9.1 (SAS Institute 2003) developed by Maia et al. (2000). Means were compared between –M and +M using Student's t -tests for independent samples.

Plant nutrient concentration

Nine whole bean plants per treatment (–M and +M) were cut off right above the first internode and RLC determined. Aboveground plant parts were pre-dried at 45°C for 48 h. C and N concentrations were determined using a LECO CHN 1000 elemental analyzer (LECO, St Joseph, Mich.). P was analyzed by a modified method according to Bowman (1989) after treatment with concentrated H_2SO_4 and subsequent determination by spectrophotometry of phosphovanadomolybdate (yellow)-molybdene blue, as described by Murphy and Riley (1962). All other nutrients were analyzed using atomic absorption spectroscopy (Thomas et al. 1967). Nutrient concentrations were compared between treatments by Student's t -tests for independent samples. Percentages were arcsine square-root transformed prior to analysis.

Results

Choice tests

Residence as well as oviposition of *T. urticae* were significantly influenced by time (binary logistic regression; for residence $Wald_1 = 10.08$, $P < 0.001$; for oviposition $Wald_1 = 5.68$, $P = 0.017$). Until 30 h after releasing the females no significant preference could be detected in residence. Beginning from 30 h after release (with the only exception of 74 h after release) significantly more females resided on the +M than on the –M side (binomial tests; $P < 0.05$ for each observation point) (Fig. 1a). A similar pattern was observed for oviposition site preference with significant preference for +M beginning 54 h after release (binomial tests; $P < 0.05$ for each observation point) (Fig. 1b). At the end of the experimental period i.e., after 78 h, a total of 16.1 ± 2.1 (SE) eggs had been laid on the +M side as compared to 6.6 ± 1.2 (SE) on the –M side (paired t -test; $t = 3.27$, $P = 0.003$).

Juvenile survival and development

None of the 22 +M and the 23 –M *T. urticae* test individuals died during the experiment. The mean overall developmental time of +M individuals (260.64 ± 2.15 SE h) was not different from that of –M individuals (264.65 ± 3.24 SE h). However, eggs laid by females that had been reared on +M leaflets prior to experiments hatched significantly earlier than eggs laid by females reared on –M leaflets (Table 1). Irrespective of treatment and life stage except the egg, developmental times of males were significantly shorter than those of females. The interaction between sex and treatment had a significant effect on developmental time of deutonymphs (Table 1).

Fig. 1 Residence (a) and oviposition site (b) preference of *Tetranychus urticae* females given a choice between leaflets from bean plants that were either not colonized (–M) or colonized (+M) by the arbuscular mycorrhizal (AM) fungus *Glomus mosseae*. Asterisks above graphs indicate a significant influence of time on choice (binary logistic regression). Asterisks beside bars indicate significant divergence from equal distribution for a given observation point (binomial tests) (* $P < 0.05$, ** $P < 0.01$)

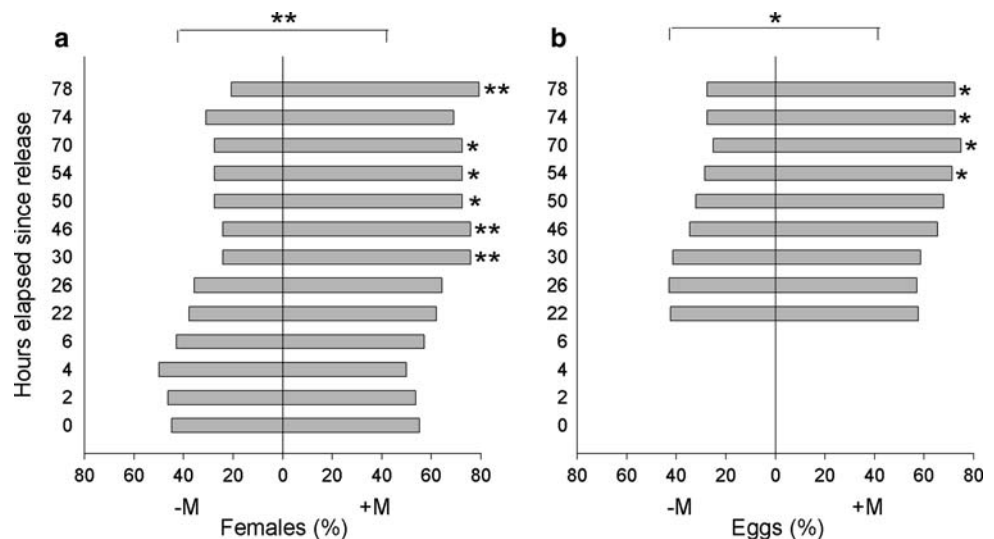


Table 1 Lifestage-specific and overall developmental time (h) (mean ± SE) of *Tetranychus urticae* held on leaflets of bean plants not colonized (–M; n = 23) or colonized (+M; n = 22) by the arbuscular

mycorrhizal fungus *Glomus mosseae*. E Egg, L larva + protochrysalis, P protonymph + deutochrysalis, D deutonymph + teliochrysalis

Treatment		E	L	P	D	Egg-adult
–M		125.87 ± 1.72	48.52 ± 1.60	36.52 ± 0.52	53.74 ± 2.12	264.65 ± 3.24
+M		121.00 ± 1.49	48.00 ± 1.37	39.82 ± 1.45	51.82 ± 1.22	260.64 ± 2.15
ANOVA ^a						
Sex	F _{1,41}	3.40	14.93	6.42	36.69	33.79
	P	0.073	0.000	0.015	0.000	0.000
Treatment	F _{1,41}	5.12	0.10	3.90	0.00	0.41
	P	0.029	0.755	0.055	0.948	0.524
Sex × treatment	F _{1,41}	0.05	2.13	3.90	6.38	1.74
	P	0.824	0.152	0.055	0.016	0.195

^a ANOVA for the influence of treatment and sex on lifestage-specific and total developmental times

Adult survival and oviposition, offspring sex ratio

A total of 30 out of 38 (79%) and 24 out of 30 (80%) females survived until the end of the experiment (14 days) for the +M and –M treatment, respectively. For the comparison of oviposition over time, only data from females that were present and alive throughout the whole 14 day period were analyzed. The pre-oviposition period (time lapse between molting to adult female and first egg laid) lasted 1–2 days and did not differ between treatments (Mann–Whitney; U = 529; P = 0.263). Repeated measures ANOVA showed a significant impact of time (F_{12,624} = 81.68, P < 0.001), treatment (F_{1,52} = 4.66, P = 0.035) and the interaction of the two sources of variation (F_{12,624} = 2.839, P = 0.001) on mean oviposition rate (Fig. 2). The latter was due to an increasing difference between oviposition rates on +M and –M leaflets in the course of the experiment. The sex ratio of offspring produced throughout the entire experimental period was slightly more female-biased (bordering significance) for +M than –M females (Table 2). During peak oviposition, between days 4 and 6, +M females produced a significantly higher percentage of female offspring than –M females.

Population growth parameters

AM symbiosis enhanced all estimated population growth parameters of *T. urticae* but the mean generation time (T) (Table 3).

Plant nutrient concentration

Zn and Mn were found in significantly smaller amounts in +M as compared to –M plants, whereas the concentrations

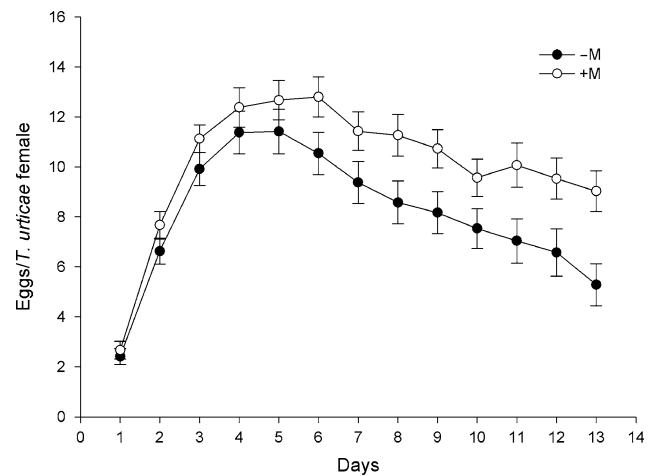


Fig. 2 Mean oviposition (±SE) of *T. urticae* females on leaflets of –M or +M plants over the first 13 days of the oviposition period. For abbreviations, see Fig. 1

Table 2 Offspring sex ratios (female proportion) of *T. urticae* females held on –M or +M leaflets in dependence of time after the onset of oviposition. Statistical values refer to pairwise χ^2 -tests within time periods. For abbreviations, see Table 1

Days	Sex ratio ^a		χ^2	P
	–M	+M		
1–3	0.47 (192)	0.46 (209)	0.15	0.386
4–6	0.51 (143)	0.65 (208)	6.74	0.007
7–9	0.61 (153)	0.58 (178)	0.29	0.335
10–13	0.46 (132)	0.51 (95)	0.41	0.306
1–13	0.51 (620)	0.55 (690)	2.02	0.086

^a Number of individuals in parentheses

of K and P were significantly increased in plants colonized by AM fungi. Neither N nor C concentrations were altered by AM symbiosis (Table 4).

Table 3 Population growth parameters (mean \pm SE) of *T. urticae* on –M or +M leaflets. R_0 Net reproductive rate, r_m intrinsic rate of increase per day, λ finite rate of increase per day, T mean generation time (days), Dt doubling time (days); for other abbreviations, see Table 1

Parameter	–M	+M	P^a
R_0	44.858 \pm 5.025	61.703 \pm 5.401	0.026
r_m	0.253 \pm 0.006	0.272 \pm 0.005	0.019
λ	1.287 \pm 0.008	1.312 \pm 0.007	0.019
T	15.092 \pm 0.105	15.188 \pm 0.071	0.452
Dt	2.744 \pm 0.067	2.551 \pm 0.469	0.022

^a t -test for independent samples using LIFETABLE.SAS (Maia et al. 2000)

Table 4 Plant nutrient contents (p.p.m. or % dry weight; mean \pm SE) of –M ($n = 9$) and +M ($n = 9$) aboveground plant parts. Statistical values refer to Student's t -tests for independent samples. Percentages were arcsine square-root transformed before analyses. RLC Root length colonized by AM fungus; for other abbreviations, see Table 1

Source of variation	–M	+M	t	P
RLC (%)	0.00 \pm 0.00	37.33 \pm 3.35		
Fe (p.p.m.)	91.32 \pm 6.45	100.52 \pm 6.61	–0.99	0.334
Mn (p.p.m.)	70.06 \pm 4.49	45.95 \pm 1.77	4.99	<0.001
Cu (p.p.m.)	7.55 \pm 0.25	8.27 \pm 0.32	–1.73	0.103
Zn (p.p.m.)	41.36 \pm 1.53	30.42 \pm 1.23	5.59	<0.001
K (%)	3.93 \pm 0.15	4.50 \pm 0.08	–3.45	0.003
Ca (%)	3.13 \pm 0.21	2.79 \pm 0.11	1.35	0.195
Mg (%)	0.48 \pm 0.03	0.46 \pm 0.01	0.54	0.598
P (%)	0.13 \pm 0.01	0.17 \pm 0.01	–3.04	0.008
C (%)	42.44 \pm 0.52	42.97 \pm 0.61	–0.65	0.524
N (%)	4.66 \pm 0.11	4.97 \pm 0.59	–0.42	0.679
C:N	9.15 \pm 0.27	9.27 \pm 0.69	–0.16	0.873

Discussion

Root colonization by the AM fungus *G. mosseae* affected host plant choice and life history performance of *T. urticae* and altered the nutrient content of its host plant. Adult *T. urticae* females preferentially fed and oviposited on +M leaflets when given a choice between +M and –M leaflets. AM symbiosis shortened egg developmental time of *T. urticae* and increased its oviposition rate and female offspring proportion during peak oviposition. The improved life history parameters resulted in enhanced population growth rates. Plant content of the micronutrients Mn and Zn was reduced whereas that of the macronutrients K and P was increased by AM symbiosis.

Due to their minute size, broad host range and limited mobility, the host plant choice of spider mites should rather be understood as host plant acceptance than host plant

finding (Sabelis 1985). The first choice made by adult female *T. urticae* in our experiment supports this assumption because an equal number of females chose to reside on +M and –M leaflets right after release. Only later on in the experiment did females more frequently reside and oviposit on +M than –M leaflets. Host plant acceptance and life history performance were positively correlated, which is a common trend in the polyphagous herbivore *T. urticae* (e.g., Yano et al. 1998).

Population growth of *T. urticae* may be influenced by various environmental factors such as temperature, humidity, plant quality and quantity, predation and inter- and intraspecific competition (e.g., Helle and Sabelis 1985). The design of our experiments delimits the aforementioned factors to plant quality only, which can be described as chemical as well as physical properties of the plant tissue. Total developmental time, one of the paramount determinants of population growth (Cole 1954), was unchanged by AM symbiosis. Spider mites are limited in minimizing developmental time due to physiological constraints (Sabelis 1985). Nonetheless, eggs on +M laid by females reared on +M leaflets hatched significantly earlier than eggs on –M laid by females reared on –M leaflets, suggesting maternal effects. Maternal provisioning of eggs has been shown to influence embryonic development of various herbivores (Bernardo 1996) and may have important consequences for population dynamics (Benton et al. 2005). However, the head start of earlier hatching larvae on +M leaflets was compensated for by faster protonymphal development on –M leaflets, which could be due to the above-mentioned physiological constraints in terms of minimizing total developmental time. Oviposition rate and offspring sex ratio are other important life history parameters of spider mites highly dependent on plant quality (Wrensch and Young 1983; Wrensch 1985). Higher oviposition rates on +M emphasize the general pattern of qualitative superiority of the +M leaf substrate for spider mite population growth. *T. urticae* is arrhenotokous, with unfertilized eggs resulting in haploid males (Wrensch 1985). Consequently, constraints in sperm availability and use influence sex allocation at the beginning of the oviposition period (e.g., Roy et al. 2003). Offspring sex ratios on +M and –M leaflets followed the general pattern of less female offspring at the onset of oviposition, yet females feeding on +M produced significantly more female offspring during peak oviposition, between days 4 and 6, than females feeding on –M, again indicating nutritional superiority of the +M leaflets.

Bean plants are widely recognized as being optimal host plants for *T. urticae*. Consequently, on both +M leaflets and –M leaflets the population growth parameters of *T. urticae* ranged under the highest values reported for this species under similar climatic conditions (Rodriguez and Rodriguez 1987). However, we still detected a notable difference

in spider mite population growth and host acceptance between plants that were colonized and plants that were not colonized by the AM fungus. This allows the conclusion that AM symbiosis induced significant, and for the spider mite detectable, changes in the quality of bean as host plant.

So far nobody has been able to conclusively pinpoint AM-induced changes in herbivore performance (positive or negative) to one—or more likely—a set of plant quality traits (Gange 2007). Colonization by AM fungi may change various plant attributes. Its effect on plant nutrient status, especially the increase in P, is undoubted (Smith and Read 1997). Gange and West (1994) established a causal link between AM-induced changes in the concentration of plant defense compounds and herbivore performance. However, information on AM-induced changes in secondary metabolite content of aboveground plant parts is scarce (e.g., Ponce et al. 2004; Khaosaad et al. 2008). In our study all measured parameters indicate the herbivore's preference for, and better performance on, colonized plants, making an increase in defensive compounds rather unlikely. Borowicz (1997) and Goverde et al. (2000) suspected AM-induced changes in the host plant nutritiousness to be responsible for altered herbivore performance. We identified changes in the concentration of plant nutrients known to influence spider mite population growth. Various studies demonstrated positive correlations between plant tissue nutrient concentrations, especially N and P, and insect herbivore growth (reviewed by Kagata and Ohgushi 2006) and particularly tetranychid mite fertility (reviewed by Rodriguez and Rodriguez 1987; Wermelinger et al. 1991). In our study, probably due to surplus N fertilization, N was not a limiting factor to test plants and was found at similar concentrations in both treatments. We therefore assume that the higher concentration of P in the aboveground plant tissue of +M plants could have been an important factor determining the differences in population growth of *T. urticae*. Egg production dissipates high amounts of P (Rodriguez 1954). Nevertheless, higher plant tissue concentrations of K as found in +M plants have been reported to be negatively correlated with the r_m value of *T. urticae* (Suski and Badowska 1975). In contrast to macronutrients, *T. urticae* appears to be quite tolerant to changes in the concentrations of micronutrients (Cannon and Terriere 1966). Altogether, our study suggests that changes in the nutritive value of parenchyma cell contents induced by AM, especially the elevated P level, could be one explanation for the enhanced population growth rates of *T. urticae*.

Different herbivore feeding guilds may be differently affected by host plant quality (Awmack and Leather 2002). Therefore, mode and site of feeding have been considered to influence the outcome of AM-induced changes in plant quality on herbivores (Gehring and Whitham 2002). *T. urticae* feeds on plant surfaces, piercing the

parenchyma cells and sucking out their contents (Tomczyk and Kropczynska 1985). Such a mode of feeding has not yet been categorized in terms of AM effects. However, there are two studies on herbivores feeding at the same site and probably ingesting similar plant fluids as *T. urticae*. Koschier et al. (2007) did not find any significant changes in oviposition or host plant preference of the polyphagous western flower thrips *Frankliniella occidentalis* Pergande, which feeds on plant surfaces by utilizing its rasping-sucking mouthparts. However, experimental thrips females were only allowed to feed and oviposit on +M leaves for 24 h, excluding preference development over time as seen in our study where the spider mite females were allowed to feed on +M leaflets for 14 days. For the Mexican bean beetle *Epilachna varivestis* Mulsant, a specialist herbivore which scrapes the leaf surface to ingest released plant juices, Borowicz (1997) assessed higher pupation rates, mass at pupation and survival until eclosion caused by AM symbiosis.

Enhanced foraging activity and population growth of *T. urticae* induced by AM symbiosis increases the loss of photosynthetic tissue and may lead to earlier destruction of the host plant. At first this is surprising assuming mutualism between AM and the host plant, because both plant and AM fungus would be negatively affected by attracting and boosting herbivory (e.g., Gange et al. 2002). However, this is only one aspect of the AM fungus-plant-herbivore interaction excluding possible compensatory mechanisms such as plant tolerance (Strauss and Agrawal 1999) or indirect defense via the natural enemies of the herbivores (Sabelis et al. 1999). Reported negative effects of mycorrhiza on herbivores have been commonly related to direct plant defense mechanisms (Rabin and Pacovsky 1985; Gange and West 1994; Vicari et al. 2002; Wamberg et al. 2003) but only two studies looked at the third trophic level (Gange et al. 2003; Guerrieri et al. 2004). The latter two studies indeed suggest that natural enemies may be a key factor rendering AM symbiosis, beneficial for either partner. To obtain a better understanding of the evolution and fitness effects of AM symbiosis, we therefore propose to investigate more thoroughly effects of AM-induced changes on plant tolerance and indirect defense mechanisms, and specifically the impacts on higher trophic levels via herbivore-induced plant volatiles (Sabelis et al. 1999) or bottom-up trophic cascades (Kagata and Ohgushi 2006).

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