

ORIGINAL ARTICLE

Root colonization by the arbuscular mycorrhizal fungus *Glomus mosseae* and enhanced phosphorous levels in cucumber do not affect host acceptance and development of *Frankliniella occidentalis*

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(Received 10 October 2006; accepted 22 January 2007)

Abstract

We tested the effect of root colonization of cucumber (*Cucumis sativus* L.) by the arbuscular mycorrhizal fungus (AMF) *Glomus mosseae* on different parameters of a plant-thrips (*Frankliniella occidentalis* Pergande) interaction. In leaf disc bioassays, the feeding activity, the oviposition rate, the settling preference of adult females and the developmental time (first instar larva to adult) on leaves of mycorrhizal and non-mycorrhizal plants were studied. To distinguish between a nutritional effect through an improved phosphorous (P) status of the mycorrhizal plant and other effects caused by mycorrhization, non-mycorrhizal plants watered with a nutrient solution with (+P) or without P (-P) were included in the study. Mycorrhization did not affect any of the parameters on host acceptance tested, whereas on plants with a higher P-level the duration of the non-feeding stages (pronymphae, nymphae) of *F. occidentalis* was shortened, but all other developmental parameters were similar as in the control and the mycorrhizal plants. Our data indicate that the polyphagous thrips *F. occidentalis* is neither affected by mycorrhization of cucumber plants nor by enhanced P-levels.

Keywords: *Arbuscular mycorrhiza*, *Cucumis sativus*, *Frankliniella occidentalis*, *Glomus mosseae*, *herbivore*, *thrips*

Introduction

Arbuscular mycorrhiza (AM) is a symbiosis between root colonizing soil-borne fungi and most land plants. Root colonization by arbuscular mycorrhizal fungi (AMF) results in a positive effect on plant growth mainly through an improved nutritional status of the host plant (Smith & Read 1997). Apart from an effect on below-ground plant-microbe interactions, it has been reported that mycorrhization also affects herbivores feeding on above-ground plant organs (reviewed by Gehring & Whitham 2002).

Variable effects of mycorrhization on leaf chewing or sap sucking herbivores have been reported. The performance of leaf chewing herbivores such as the generalist lepidopteran species *Artia caja* L. (Gange & West 1994), *Heliothis zea* (Boddie) and *Spodoptera frugiperda* Smith (Rabin & Pacovsky 1985) has been reported to be negatively affected by mycorrhization of their host plants. In contrast, Gange et al. (2005) found leaf-mining generalists to respond positively to AM colonization. Other

generalists such as grasshoppers feeding on tallgrass prairie plants showed no preference for plants with or without mycorrhizal fungi (Kula et al. 2005). Goverde et al. (2000) reported that the survival and larval weight of the leaf chewing lepidopteran specialist *Polyommatus icarus* Rottemburg was positively affected by mycorrhization of its host plant *Lotus corniculatus* L. (Fabaceae). Reports on the effects of mycorrhization on phloem sap feeders range from positive to negative. While other life history traits were unaffected, mycorrhizal colonization exhibited a positive effect on traits such as adult weight and fecundity of the generalist aphid species *Myzus ascalonicus* Doncaster and *M. persicae* (Sulzer) (Gange et al. 1999), whereas no effect on the reproductive behaviour and feeding of the generalist *Schizaphis graminum* (Rondani) (Pacovsky et al. 1985) was found. In contrast, the development and the reproduction rate of the generalist *Macrosiphum euphorbiae* (Thomas) was significantly lower on arbuscular mycorrhizal tomato plants compared to non-mycorrhizal plants (Guerrieri et al. 2004). Gehring and Whitham (2002) examined the

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performance of the specialist phloem-feeding aphid *Chaitophorus populicola* Thomas on cottonwood (*Populus angustifolia* × *P. fremontii*) and found that the aphid population was higher on plants without mycorrhizal colonization.

To our knowledge there is only one study on the effect of mycorrhization on insects feeding on parenchyma cells of leaves. Borowicz (1997) reported that the performance of the Mexican bean beetle *Epilachna varivestis* Mulsant, a coleopteran species with a narrow host range, was positively affected when reared on mycorrhizal soybean plants.

No data are yet available on the effect of mycorrhization on the highly polyphagous thrips species *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae), the western flower thrips. Adults and larvae of thrips puncture sub-epidermal cells of green leaves and suck out the contents (Tommasini & Maini 1995).

In the present study we tested whether parameters of the plant-thrips interactions such as the settling preference, the damage caused by feeding, the oviposition rate and the developmental time of the larvae of *F. occidentalis*, feeding on leaves of cucumber (*Cucumis sativus* L.) are affected by root colonization by the AMF *Glomus mosseae*. To distinguish between a nutritional effect through an improved phosphorous (P) status of the mycorrhizal plant and other effects caused by mycorrhization, non-mycorrhizal plants watered with a nutrient solution with (+P) or without P (-P) were included in the study.

Materials and methods

Biological material

Inoculum production and plant inoculation. The AMF inoculum was produced in an autoclaved (20 min; 121°C) mixture of silicate sand, expanded clay and soil (1:1:1; by vol.) where beans were grown in presence of sporocarps of *Glomus mosseae* (Nicolson & Gerdemann) Gerd. & Trappe (BEG 12; European Bank for the Glomales) for 3 months. The AMF inoculum consisted in the growth substrate and a mixture of sporocarps, spores, hyphae and infected root pieces.

For the experiments cucumber (*Cucumis sativus* L., var. "Korinda F1") seeds were surface-sterilised in 50% commercial bleach for 5 min, rinsed three times in sterile distilled water and germinated in autoclaved (20 min; 121°C) perlite. Eight days after seeding the plants (one plantlet/pot; pot diameter 10 mm with a volume of approx. 750 ml) were transferred into the substrate described above. For inoculation a hole was made into the growth substrate and approx. 5 g of inoculum were added to the hole. Plants were planted into this hole, thus roots had to traverse the inoculum and became colonized.

Plant nutritional treatments. All plants were grown in the greenhouse and watered with a nutrient solution with P (+P) or without P (AMF, -P). The nutrient solution without P consisted of Ca(NO₃)₂ 0.472 g/l, K₂SO₄ 0.256 g/l; MgSO₄ 0.136 g/l; MoO₃ 0.07 g; NH₄NO₃ 8 mg/l; Fe₆H₅O₇ × 3 H₂O 50 mg/l; Na₂Bo₄O₇ × 4H₂O 1.3 mg/l; MnSO₄ × 4 H₂O 1.5 mg/l; ZnSO₄ × 7 H₂O 0.6mg/l; CuSO₄ × 5 H₂O 0.54 mg/l; Al₂(SO₄)₃ 0.028 mg/l; NiSO₄ × 7 H₂O 0.028 mg/l; Co(NO₃)₂ × 6H₂O 0.028 mg/l; TiO₂ 0.028 mg/l; LiCl₂ 0.014 mg/l; SnCl₂ 0.014 mg/l; KJ 0.014 mg/l; KBr 0.014 mg/l. To the nutrient solution with P additionally KH₂PO₄ (0.136 g/l) was added.

At the end of the experiment the shoot fresh weight, the degree of AM root colonization and the P-content in the leaves was determined.

Estimation of root colonization. To visualize the AMF colonization, roots were cleared by boiling 4 min in 10% KOH, rinsed three times with tap water and stained according to the method of Vierheilig et al. (1998) by boiling for 4 min in a 5% ink (Shaeffer; jet-black)/household vinegar (=5% acetic acid) solution. After staining, the percentage of root colonization was determined according to the method of Newman (1966).

Assessment of P-status. The P-content in dried leaves was determined with the ammonium-vanadatomolybdenum method (Gericke & Kurmies 1952). Values are given as percentage of P of plant dry weight.

***F. occidentalis* rearing.** The *F. occidentalis* rearing was maintained in a climate chamber at 24 ± 1°C and 60 ± 5% relative humidity with a photoperiod of 16:8 (L:D) h. Cucumber (*C. sativus* L., var. "Korinda F1") plants were grown routinely to maintain the *F. occidentalis* colony. About 100 adult females were allowed to lay eggs on detached cucumber leaves on 1% water agar in plastic Petri dishes (diameter 14.0 cm). Petri dish lids with central holes covered with a fine mesh to ensure ventilation were fixed on the dish bottom with Nesco® sealing film (Azwell Inc., Osaka, Japan) to prevent thrips from escaping. After 48 h, the thrips were removed and the leaves in the Petri dishes were kept in the climatic chamber until emerged larvae had developed into larvae, nymphae or adults. Larvae, nymphae or adults were used in bioassays or adults were collected from these units and isolated on fresh leaves on agar in separate Petri dishes to continue the rearing.

Leaf disc bioassays

General leaf disc assay procedure. In all bioassays, the youngest leaves from potted cucumber plants (plant age around 7–8 weeks) of the respective treatment (AMF, +P, -P) were punched using a cork borer to

obtain leaf discs (diameter 2.0 cm). All bioassays were conducted in a climate chamber at $24 \pm 1^\circ\text{C}$ and $60 \pm 5\%$ relative humidity with a photoperiod of 16:8 (L:D) h.

Assessment of feeding damage. Leaf discs taken from the respective treatment (AMF, +P, -P) were placed singly in glass Petri dishes (diameter 6.0 cm) on a 1% water agar (Fluka® Sigma Aldrich Chemie GmbH, Steinheim, Germany) film. Five adult *F. occidentalis* females of unknown age were collected randomly from the rearing and introduced into these bioassay units. Each unit was covered with a thin plastic film (Toppits®, Melitta Bentz KG, Minden, Germany), which was perforated (one hole per cm^2 on average) by means of insect pins (diameter 0.4 mm) for ventilation. After 24 h, thrips were removed and the percentage of silver damage on the cucumber leaf discs was measured using an image analysis system that consisted of a binocular microscope, a digital camera (Nikon Coolpix 2500, Nikon Co-operation, Tokyo, Japan) and an image analyzing software (Lucia G®, LIM Laboratory Imaging s.r.o., Prague, Czech Republic) (Koschier et al. 2002). Each experimental series consisted of 15 replicates.

Oviposition rate. To obtain evenly aged females, thrips pupae were collected from the rearing units and were isolated on fresh cucumber leaves on a thin 1% water agar film in separate Petri dishes (diameter 9.0 cm). Lids with condensation holes covered with fine mesh (80 μm) were fixed on the dish bottom with Nesco® sealing film. They were checked daily for adult emergence, and again emerged females were isolated on fresh leaves in similar units. These females (48 h post-emergence) were allowed to oviposit singly on leaf discs taken from the respective treatment (AMF, +P, -P) on moist filter paper in small glass Petri dishes (diameter 6.0 cm). Each unit was covered using a thin plastic film as described above. After 24 h, the number of eggs on each leaf disc was determined using a binocular microscope. Each experiment was replicated 20 times per treatment.

Development time. To obtain synchronized cohorts of even-aged thrips larvae, females of unknown ages collected randomly from the rearing were allowed to oviposit on cucumber leaves on 1% water agar in Petri dishes for 6 h. After four days newly emerged larvae were isolated singly on the leaf discs from the respective treatment (-P, AMF, +P) on 1% water agar in small Petri dishes (6 cm diameter). Each unit was covered with a thin plastic film as described above. During the following 10 days the stage of larval development was recorded at 24-h intervals until each individual died or became an adult. Each experiment was replicated at least 20 times per treatment.

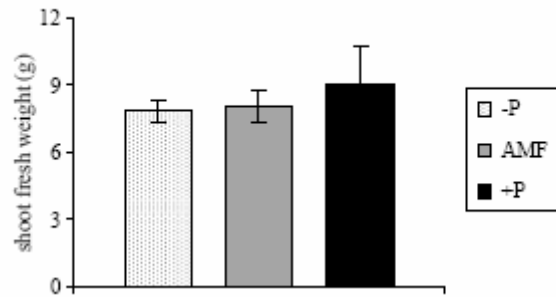


Figure 1. Shoot fresh weight (mean \pm SE) of plants inoculated with the AMF (AMF) or non-inoculated but with P applied (+P) or not applied (-P). Means are not significantly different - n.s. ($p > 0.05$); One-way ANOVA.

Settling experiments. In a three-choice bioassay leaf discs of all three treatments (AMF, +P, -P) were placed at equal distances from each other in a glass Petri dish bottom on moist filter paper. Thrips females were released singly in the centre of the triangle formed by the position of the leaf discs. Thrips position was recorded in 60-min intervals for 6 h. Each experiment was repeated 40 times.

Statistical analysis

Data were analysed using SPSS for Windows 11.0. Homogeneity of variances was tested using Levene's test. A One-Way-ANOVA was performed to determine differences between percentage of damaged area or time spent settled on the leaf discs, development time, shoot fresh weight or oviposition rate of each treatment, respectively. Means were compared using Fisher's least significant difference ($\text{LSD}_{\alpha 0.05}$).

Results

The degree of root colonization in the mycorrhizal cucumber plants (AMF treatment) reached $65 \pm 5\%$. The shoot fresh weight of all plants, whether inoculated with the AMF or P-applied, showed no significant differences (Figure 1). The assessment of the P-status of the cucumber plants confirmed that plants watered with nutrient solution containing phosphorous (+P-treatment) showed the highest

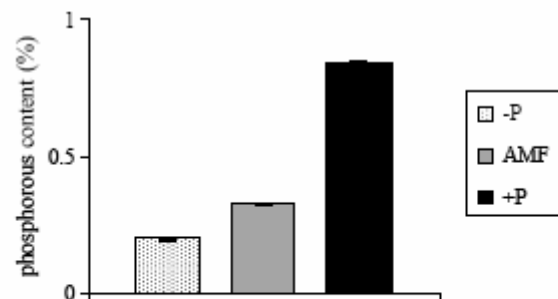


Figure 2. Leaf phosphorous content (mean \pm SE) of plants inoculated with the AMF (AMF) or non-inoculated but with P applied (+P) or not applied (-P), mixed leaf samples.

Table I. Percentage of damaged area (mean value \pm SE) on leaf discs from plants treated with AMF or without (-P, +P) caused by *F. occidentalis* females. n.s. - non significant ($p > 0.05$); One-way ANOVA.

Treatment	Percentage of damaged area (mean value \pm SE)
-P	66.02 \pm 0.99
AMF	66.49 \pm 0.92
+P	54.19 \pm 0.48
F-ratio	0.71 n.s.
df	2, 42

P-level, AMF plants an intermediate P-level, whereas -P control plants had the lowest P-level (Figure 2).

There was a tendency that the feeding activity of *F. occidentalis* females was slightly lower and thus resulted in less damage on the leaf discs from plants of the +P-treatment, though this effect was not significant (Table I).

A slight preference of *F. occidentalis* females to oviposit on leaf discs from plants watered with nutrient solution containing phosphorous was observed, however this effect was not significant (Figure 3). Compared to the oviposition rate on leaf discs from the +P-treatment about 15% and 30% less eggs were laid on leaf discs from the AMF-treatment and the -P-treatment, respectively.

While the duration of the larval *F. occidentalis* feeding stages was not influenced by any treatment, non-feeding stages on leaf discs from plants treated with the +P nutrient solution developed significantly faster (by about 10 h) than on leaf discs from plants watered with the -P nutrient solution (Table II). However, the total development time of *F. occidentalis* was similar in all treatments, thus, excluding an effect of the phosphorous content of the cucumber leaves on the total development time.

Though the differences are not statistically significant, when they had a choice, the thrips females spent about 17% more time on leaf discs from +P-treatment compared to the -P-treatment and about 6% more time on leaf discs from plants inoculated with AMF (Figure 4).

Table II. Development time (hours) of feeding stages (larvae 1, larvae 2), non-feeding stages (pronymphae, nymphae) and total development time (larvae 1 - adults) of *F. occidentalis* individuals on leaf discs from plants treated with AMF or without (-P, +P). Means are significantly different at $p \leq 0.05$ (*); n.s. - non significant ($p > 0.05$); One-way ANOVA, last significant difference (LSD $_{\alpha 0.05}$).

Treatment	n	Duration feeding stages (mean value \pm SE)	Duration non-feeding stages (mean value \pm SE)	Total development time (mean value \pm SE)
+P	21	134.86 \pm 6.08	73.14 \pm 3.50 b*	208.00 \pm 5.58
AMF	28	120.00 \pm 3.49	80.57 \pm 2.21 a b	200.57 \pm 3.54
-P	24	130.00 \pm 5.94	83.00 \pm 2.49 a	213.00 \pm 6.82
F-ratio	-	2.26 n.s.	3.33*	1.48 n.s.
df	-	2, 70	2, 70	2, 70

*Mean values followed by the same letter are not significantly different.

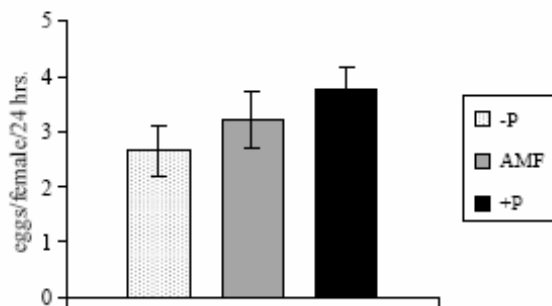


Figure 3. Oviposition rate (mean number of eggs/female/24 h \pm SE) on leaf discs from plants treated with AMF or without (-P, +P). Means are not significantly different - n.s. ($p > 0.05$); One-way ANOVA.

Discussion

Feeding of herbivores on mycorrhizal and non-mycorrhizal plants has been reported in a number of studies ranging from positive to negative effects on the herbivore (Gehring & Whitham 2002). This is the first report on how mycorrhization in cucumber plants affects different parameters of the plant-*F. occidentalis* interaction. In our study mycorrhization did not exhibit a clear effect on any of the behavioural parameters of *F. occidentalis*. The feeding activity, the oviposition rate and the settling preference, all indicators for changes in the host plant affecting the behaviour of *F. occidentalis*, were always similar on leaves of mycorrhizal and non-mycorrhizal cucumber plants. Thrips females showed a slight non-significant tendency to prefer leaves with a high phosphorous content. A similar effect was reported by Chen et al. (2004) with potted *Impatiens walleriana* plants. They found a strong trend towards increased *F. occidentalis* population growth on plants with higher foliar P content.

Physiological qualitative/quantitative changes in the plant can affect its nutritional value and thus can result in developmental changes of the plant feeder. Looking at the developmental time of the larvae of *F. occidentalis* feeding on mycorrhizal and non-mycorrhizal cucumber plants we observed no differences, indicating that changes in the mycorrhizal plants had no nutritional effect on *F. occidentalis*. In P-treated cucumber plants the duration of the non-feeding stages was significantly shorter

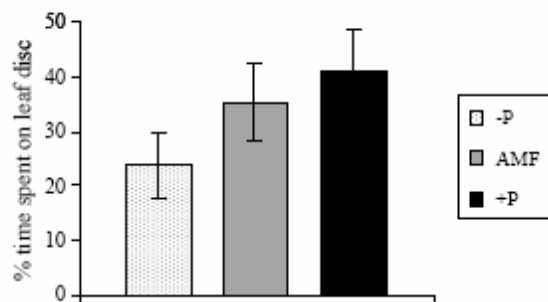


Figure 4. Percentage of time *F. occidentalis* females spent settled when given a choice within a maximum time span of 6 h. Means are not significantly different - n.s. ($p > 0.05$); One-way ANOVA.

compared to the -P plants. This could indicate that a P-treatment of the plant could alter the level of compounds in the plant taken up by the larvae and thus, stimulating certain developmental stages of *F. occidentalis*. However, this development-stimulating effect had disappeared when looking at the total development time, thus being without any obvious importance for the general development.

These data seem to contradict observations with another sub-epidermal cell feeder of leaves, the Mexican bean beetle. The mass of Mexican bean beetle at pupation and the pupation rate was enhanced after feeding on leaves of mycorrhizal soybean plants, whereas feeding on leaves of P-supplied plants showed no effect on the beetle (Borowicz 1997). However, this apparently contrasting effect of the two sub-epidermal cell feeders *F. occidentalis* and *E. varivestis* might be due: (i) To different feeding techniques, *F. occidentalis* punctures the sub-epidermal cell, whereas *E. varivestis* feeds by slashing cells and lapping up the contents, (ii) to different test plants, or (iii) to the fact that generalist and specialist herbivores do not react similarly to physiological changes of the host plant.

To our knowledge the absence of an effect of mycorrhization on a sap sucking herbivore has so far only been reported with the generalist aphid species *S. graminum*, a phloem sap feeder (Pacovsky et al. 1985), whereas other generalist phloem sap feeders such as *M. ascalonicus*, *M. persicae* and *M. euphorbiae* were positively (Gange et al. 1999) or negatively (Guerrieri et al. (2004) affected by mycorrhization. However, no data were available yet on the effect of mycorrhization on herbivores such as Thysanoptera not feeding on the phloem sap but on the sap of sub-epidermal cells of leaves (Tommasini & Maini 1995).

To summarize, this is the first report on the interaction between mycorrhizal plants and thrips. Our data indicate that neither mycorrhization nor enhanced P-levels in cucumber plants affect the interaction between plants and *F. occidentalis* and thus should not exhibit any effect on *F. occidentalis* population in nature. Further studies are needed to elucidate whether the observed phenomena, an absence of any effect of mycorrhization on behaviour

and development, is specific for *F. occidentalis* or is a more general symptom for sap feeders puncturing sub-epidermal cells.

Acknowledgements

This work was partially funded by a grant of the Thai government to TK.

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