Plant Pathology (2008) 57, 1109–1116 Doi: 10.1111/j.1365-3059.2008.01895.x

Effect of *Glomus mosseae* on concentrations of rosmarinic and caffeic acids and essential oil compounds in basil inoculated with *Fusarium oxysporum* f.sp. *basilici*

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This study investigated the potential of the arbuscular mycorrhizal (AM) fungus *Glomus mosseae* to protect basil (*Ocimum basilicum*) against *Fusarium oxysporum* f.sp. *basilici* (*Fob*). It was hypothesised that *G. mosseae* could confer a bioprotective effect against *Fob* as a result of increases in leaf rosmarinic (RA) and caffeic acids (CA) or essential oil concentrations. *Glomus mosseae* conferred a bioprotective effect against *Fob* by reducing plant mortality to 20% compared to 33% in non-mycorrhizal (NM) plants. This bioprotective effect was not related to improved phosphorus (P) nutrition, as AM and NM plants treated with *Fob* had similar shoot P concentrations (6 and 8 mg g^{-1} dry weight (DW), respectively). Both AM and NM plants treated with *Fob* had similar leaf and root RA and CA concentrations. Furthermore, phenolic (40–70 mg CA g^{-1} DW) or essential oil concentrations (0·1–0·6 mg g^{-1} DW) were not increased in plants treated with the AM fungus and *Fob*. Therefore, the bioprotective effect conferred by *G. mosseae* was not a result of increases in the phytochemicals tested in this study. However, under the AM symbiosis, basil plants treated with *Fob* had lower methyleugenol concentrations in their leaves (0·1 mg g^{-1} DW) than NM plants treated with the pathogen (0·6 mg g^{-1} DW).

Keywords: arbuscular mycorrhizas, bioprotection, *Fusarium oxysporum*, *Ocimum basilicum*, phytochemicals

Introduction

Basil (*Ocimum basilicum*, Lamiaceae), also known as sweet basil, is widely appreciated for its culinary uses and medicinal properties. These properties are mainly attributed to its essential oil composition, but also to other antioxidants it contains, such as rosmarinic acid (RA) and caffeic acid (CA). The antioxidants are also known to act as defence compounds, which are found generally within the Lamiaceae. Basil has high concentrations of two major essential oil compounds, eugenol and linalool (Simon, 1998; Jirovetz *et al*., 2003), which have a variety of applications as ingredients in foods and as flavours (Sacchetti *et al*., 2004). These two compounds were also shown to have high antimicrobial activity against bacteria such as *Escherichia coli* and *Lactobacillus plantarum* (Bouzouita *et al*., 2003). Basil is grown as a major commercial crop in several countries, including Greece, France, Israel and Italy (Swart & van Niekerk, 2003; Biris *et al*., 2004).

In the last decade, there have been several reports of an increasing occurrence of fusarium wilt, which causes serious damage to basil production (Gamliel *et al*., 1996; Fravel & Larkin, 2002; Swart & van Niekerk, 2003; Biris *et al*., 2004; Moya *et al*., 2004). More specifically, *Fusarium oxysporum* f.sp. *basilici* (*Fob*) was reported to be the major disease that limits the production of this crop worldwide (Gamliel *et al*., 1996). The symptoms associated with *Fob* include chlorosis, necrosis, wilt of the stems and leaves, crown and root rot, dark lesions and vascular discoloration, all of which often lead to plant death. As basil is an economically important crop for many countries, efforts have been made to find ways of reducing the damage done by *Fob*. Better management practices to improve resistance of basil to *Fob* include seed

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Published online 18 June 2008

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sterilization, crop rotation, soil sterilization, crop breeding and selection, and use of fungicides during plant growth. For basil and many other herb crops, the use of pesticides is undesirable because of consumer preferences. In this light, better and more efficient methods of controlling *Fob* and increasing basil resistance to this pathogen need development.

As with most species belonging to the Lamiaceae, basil is known to form arbuscular mycorrhizas (AM) (Wang & Qiu, 2006). AM fungi (AMF) colonizing plant roots are widely known for their beneficial effects on plant growth, mainly through improved phosphorus (P) nutrition (Smith & Read, 1997) and the tolerance they provide to certain diseases (notably caused by some subspecies of *F. oxysporum*) (St-Arnaud *et al*., 1995; Fillion *et al*., 1999; Smith *et al*., 2003). AM could therefore help to reduce the negative effects of *Fob* on basil and/or increase its resistance to the pathogen. Such resistance might be attributed to the direct effect of the AM symbiont on *Fob* or indirect effects through improved nutrition or higher production of active/defence compounds, as previously shown for basil (Copetta *et al*., 2006; Toussaint *et al*., 2007) and other medicinal herbs (Kapoor *et al*., 2002a,b; Khaosaad *et al*., 2006). The present study had two main objectives: (i) to evaluate the potential of AMF to reduce the disease severity of *Fob* in basil, and (ii) to evaluate the interaction between AM and *Fob* to increase the production of RA, CA and essential oil compounds in leaves of basil, which are the harvested product.

Materials and methods

The experiment was conducted from September to October 2006 (autumn, northern Hemisphere) in Vienna, Austria. There were five treatments with 15 replicates each: (i) non-mycorrhizal (NM) plants as controls, (ii) NM plants with added phosphorus (NM + P), (iii) NM plants with *Fob* (NM + *Fob*), (iv) AM plants, and (v) AM plants with *Fob* (AM + *Fob*). These treatments allowed the ability of the AM to reduce the harmful effects of *Fob* on basil through mycorrhiza formation to be assessed, but also took into account any simple P-mediated effects (NM + P treatment) on RA, CA and essential oil accumulation.

Seeds of basil (*Ocimum basilicum* cv. Genovese, Yates Seed Ltd) were surface-sterilized by soaking in a 30% hypochlorite solution (commercial bleach 3·8%) for 5 min, rinsed with distilled water and germinated in Perlite in a controlled-temperature room (28°C). When the seedlings were 14 days old, they were transferred into small free-draining pots (8 cm diameter × 7 cm high) containing a mixture of sand, expanded clay and soil (1:1:1; v/v/v; autoclaved for 20 min at 121°C). They were planted into holes in the substrate where the AM inoculum (5 g per pot) had previously been added. The inoculum was obtained from Biorize and consisted of dried root pieces containing fungal hyphae and spores of *G. mosseae* (BEG 12; International Bank of Glomeromycota; http://www.kent.ac.uk/bio/beg/), mixed with a carrier material (silica sand and expanded clay). NM and NM + P plants received 5 g of the sand, clay and soil mixture instead of the inoculum.

Fusarium inoculation

Fusarium oxysporum f.sp. *basilici* (*Fob*) cultures were obtained from Dr M. Pasquali, University of Torino, Italy. The stock cultures of *Fob* were subcultured on potato dextrose agar (PDA, Sigma Aldrich) in Petri dishes (39 g PDA L^{-1} distilled water) for 7 days at 24°C in the dark before using them for inoculation on the plants. *Fob* inoculum was prepared by washing the dishes with sterile water, which was then filtered through cheesecloth to remove any mycelium and obtain only conidia (spores). The resulting spore suspension was examined under a light microscope to determine the number of conidia and the final concentration was adjusted to $2{\cdot}1 \times 10^6$ spores mL^{-1}. This suspension was used as the pathogen inoculum. On the day of application of *Fob*, the roots of the various plant treatments (5 weeks old) were washed and the degree of AM colonization was determined in five plants which had been inoculated with *G. mosseae* (colonization: 60 ± 7%). The roots of the plants in the *Fob* treatments were dipped in the *Fob* suspension, whereas roots of all other plants were dipped in sterile water. The plants were then transplanted into new pots containing the sand, clay and soil substrate, as previously described. The transplanting was performed for all treatments, and the plants that were mycorrhizal did not receive more inoculum as they were already well colonized.

Plant growth

Treatments were randomly arranged in a glasshouse and grown for another 5 weeks (day/night cycle: 16 h 22°C/ 8 h 19°C; relative humidity 50–70%; average light intensity 400 μmol m^{-2} s^{-1} with ~200 μmol m^{-2} s^{-1} additional lighting provided by Radium HRI-T4W/DH lamps) and watered regularly with a nutrient solution with or without P, according to treatment. The nutrient solution without P consisted of (mg L^{-1}): $Ca(NO_3)_2$, 472; K_2SO_4, 256; $MgSO_4$, 136; MoO_3, 70; NH_4NO_3, 8; $Fe_6H_5O_7{\cdot}3H_2O$, 50; $Na_2B_4O_7{\cdot}4H_2O$, 1·3; $MnSO_4{\cdot}4H_2O$, 1·5; $ZnSO_4{\cdot}7H_2O$, 0·6; $CuSO_4{\cdot}5H_2O$, 0·54; $Al_2(SO_4)_3$, 0·028; $NiSO_4{\cdot}7H_2O$, 0·028; $Co(NO_3)_2{\cdot}6H_2O$, 0·028; TiO_2, 0·028; $LiCl_2$, 0·014; $SnCl_2$, 0·014; KI, 0·014; KBr, 0·014. The nutrient solution with P consisted of the same solution with added KH_2PO_4 (0·136 g L^{-1}).

Plants were monitored regularly in the first days after application of *Fob* and mortality was recorded throughout the experiment. Plants were harvested 5 weeks after inoculation with *Fob* (10 weeks old), shoot and root fresh weight were recorded, then after oven-drying at 45°C for 3 days, the following parameters were recorded: shoot (leaf and stem) P concentration, leaf (no stem) and root RA and CA concentrations, leaf essential oil composition and concentrations, leaf total phenolic concentration and total antioxidant activity (see below for methods). Disease

severity was visually assessed by determining the mortality rate and estimating the percentage of root browning/decay.

At harvest, roots and shoots were separated, washed and blotted, and root samples were taken for the determination of *Fob* symptoms and AM colonization. Root segments (~1 cm) were cut for determination of *Fob* severity and AM colonization. The remaining root material was dried for further analysis; the percentage of root browning caused by *Fob* was estimated according to the magnified intersection method of McGonigle *et al.* (1990) by observing 100 intersections between root and ocular cross-hair for presence or absence of *Fob* lesions and root browning. The ink and vinegar staining method of Vierheilig *et al.* (1998) was used for determination of AM colonization. Briefly, root segments were cleared in 10% KOH for approx. 4 min then stained in a 5% ink/vinegar solution (Jetblack Sheaffer ink) for 4 min at 90°C. Root segments were washed several times with tap water and the degree of AM colonization was determined according to the method of McGonigle *et al.* (1990).

Phosphorus concentrations were determined using the ammonium-vanadate-molybdate method of Gericke *et al.* (1952) on pooled leaf samples as not enough plant material was available to conduct all analyses on all samples.

Chemicals for HPLC and total phenolics determination

Folin-Ciocalteu phenol reagent was obtained from Merck. DPPH (2,2-diphenyl-1-picrylhydrazyl), caffeic acid and (R)-(-)-Carvon were purchased from Sigma Aldrich. Trolox (6-hydroxy-2,5,7,8-tetra-methylchromane-2-carboxylic acid) was obtained from Fluka and rosmarinic acid from Roth. Solvents for extract preparation were of analytical grade and solvents for HPLC analysis of HPLC-grade.

Sample extraction

Samples of 100 mg dried ground leaves or roots (RA and CA) were extracted with 16 mL MeOH/water (50:50, v/v) at room temperature for 1 h in a sonicator. The filtered methanolic sample extracts were used for determination of total phenolic content, antioxidant activity and HPLC analysis. An additional 100 mg dried leaves were extracted for 30 min in a sonicator with 2 mL dichloromethane containing Carvon (0·1 μL mL^{-1}) as an internal standard. The samples were filtered and subsequently used for GC analysis.

Determination of total phenolic content

The total phenolic content was determined according to the Folin-Ciocalteu method (Singleton *et al.*, 1999). Sample extract (200 μL) were added to a flask which contained 0·5 mL Folin-Ciocalteu reagent and 10 mL water. After 3 min, 1 mL of a saturated Na_2CO_3 solution was added, vortexed and topped up with water to a final volume of 25 mL. After incubation for 1 h in the dark, the colorimetric reaction was recorded with a spectrophotometer (Hitachi Ltd) at 725 nm. The total phenolic content of the samples was expressed in mg CA g^{-1} dry weight (DW).

Determination of total antioxidant activity

Radical scavenging activity was measured using the stable radical DPPH. Briefly, 50 μL sample and 950 μL methanol were added to 1 mL DPPH (0·015% v/v in methanol). After 30 min incubation in the dark, the reductive discoloration of the DPPH radical, caused by the active antioxidants found in the sample, was recorded. Measurements were made using a spectrophotometer (Hitachi Ltd) at 515 nm, and the results were expressed in Trolox equivalents.

HPLC analysis

The method was adapted from that of Cuvelier *et al.* (1996). The analysis was performed on a reversed phase C_{18} Symmetry® column (4·6 × 150 mm, 5-μm pore size; Waters) equipped with a guard column of the same stationary phase (Symmetry® C_{18}). The mobile phase was programmed with a linear gradient from 90% A (840 mL deionised water with 8·5 mL acetic acid and 150 mL acetonitrile), 10% B (MeOH) to 100% B in 30 min, with a flow rate of 1·5 mL min^{-1}. Methanolic samples (20 μL) were injected and detection was by a photodiode array detector (Waters, 996 PDA) at 330 nm.

Essential oil analyses

Identification of the essential oil compounds was performed on a GC/MS HP 6890 chromatograph coupled to a 5972 MSD (Hewlett Packard) and fitted with a DB-5MS (30 m × 0·25 mm, 0·25-μm film thickness, Agilent). Operating conditions were helium as the carrier gas (average velocity 42 cm s^{-1}), an injection temperature of 250°C and a split ratio of 20:1. The temperature programme was 60°C for 4 min and 60–280°C at 5°C min^{-1}. The compounds were identified by comparison of retention indices (Adams, 2001) and mass spectra from the Wiley database (McLafferty, 1989). Quantification of the compounds was performed on an Agilent GS/FID (flame ionization detector).

Statistical analyses

The effects of treatments were determined by analyses of variance (ANOVAS) and differences between treatments were determined using Tukey's pairwise comparison test at a significance level of 95% (SYSTAT 11®). Data were treated in order to meet the assumptions of ANOVA (i.e. normality of data and evenness of variance) and transformations – natural log, arcsin (for percentage colonization) – were performed when required.

Results

After 10 weeks' growth, NM and NM + P plants were not colonized by the AM fungus nor infected by *Fob*. Percentage of AM colonization in plants inoculated with *G. mosseae* alone (AM) was 58%, whereas that of AM + *Fob* plants was reduced to 40% (Table 1). There was no mortality or root browning in plants not inoculated with *Fob*. The mycorrhizal status of the plants affected the disease severity of *Fob* (Table 1); a third of NM + *Fob* plants died, compared to 20% for AM + *Fob* plants. NM + *Fob* plants also had significantly higher levels of root browning (19%) than AM + *Fob* plants (6%) under the same conditions.

Additional P had no significant effect on the growth of NM plants, but inoculation with *G. mosseae* significantly increased both shoot and root dry weights by approximately 60% (Fig. 1). Inoculation with *Fob* significantly reduced shoot growth, but this effect although reduced in AM plants, was not significant Shoot P concentrations were similar in most treatments (Fig. 2). NM and AM plants had similar shoot. P concentrations (4 mg g^{-1} DW), while NM + P plants appeared to have slightly, but not significantly, higher concentrations (6 mg g^{-1} DW). NM plants treated with *Fob* had a mean shoot P concentration of 8 mg g^{-1}, which was twice as much as AM plants. Shoot P content was significantly lower in NM plants (1·2 mg) than NM + P (2·0 mg) and AM plants (2·0 mg; Fig. 1c). Inoculation with *Fob* did not affect shoot P content significantly compared to any other treatments.

Leaf RA concentrations were significantly affected by the treatments (Table 2). NM plants had the highest mean leaf RA concentration (16·8 mg g^{-1} DW), but not significantly higher than NM + P and AM plants (15·2 and 15·7 mg g^{-1} DW, respectively). NM + *Fob* plants had significantly lower RA concentrations (11·0 mg g^{-1} DW) than most other treatments. Mean leaf CA concentrations were lower than RA concentrations (0·50–0·75 mg g^{-1} DW), but there were no significant differences between treatments (Table 2). Leaf phenolic concentration was significantly higher in NM plants (69·48 mg CA g^{-1} DW) than in other treatments (41·26–55·22 mg CA g^{-1} DW), except AM plants (60·56 mg CA g^{-1} DW; Table 2). Leaf antioxidant activity was also significantly higher in NM plants than in all other treatments (Table 2). Antioxidant activity was significantly correlated with leaf RA concentration in all treatments ($R^2 = 0·41$; $P = 0·001$). There was also a significant relationship between leaf RA and phenolic concentrations for all treatments ($R^2 = 0·55$; $P \leq 0·001$). Root RA concentrations were higher than leaf RA concentrations (23·9–37·8 and 11·0–16·8 mg g^{-1} DW, respectively). Both NM + P and AM plants had significantly higher root RA concentrations than other treatments (Table 3). Root CA concentrations were slightly lower than those observed in the leaves (0·33–0·44 and 0·50–0·75 mg g^{-1} DW, respectively). AM plants had significantly ($P \leq 0·001$) lower root CA concentrations

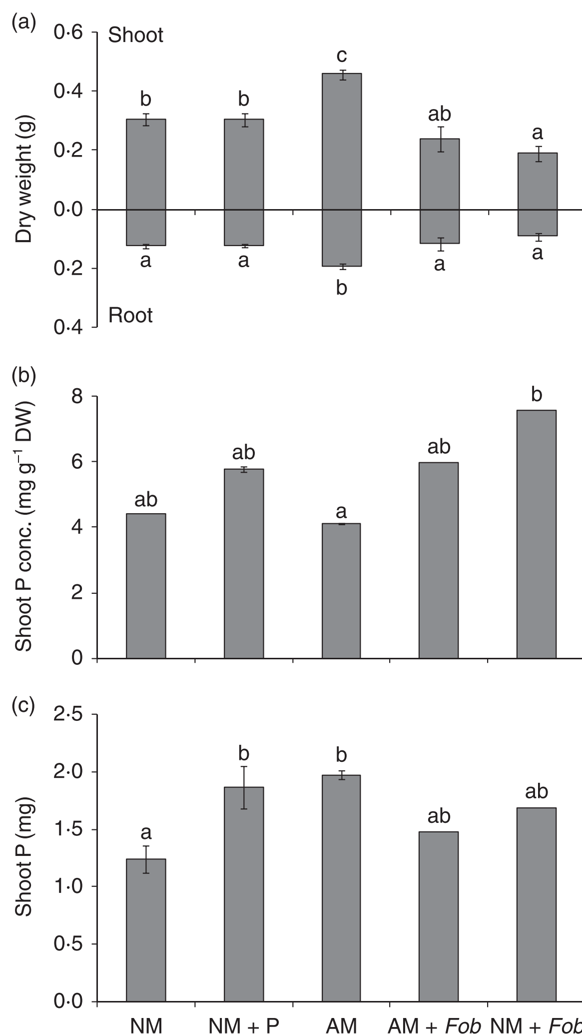

Figure 1 (a) Shoot and root dry weights (g), (b) shoot P concentration (mg g^{-1} DW), and (c) shoot P content (mg) in *Ocimum basilicum* grown under various treatments. Means (n = 10–15 for (a); for (b) and (c) n = 3, pooled samples) and SE bars are shown. Different letters represent significant differences according to Tukey's test ($P \leq 0·05$). NM, non-mycorrhizal plants; P, phosphorus-treated plants; AM, mycorrhizal plants inoculated with *G. mosseae*; *Fob*, plants treated with *Fusarium oxysporum* f.sp. *basilici*.

Table 1 Percentage arbuscular mycorrhizal (AM) colonization, plant mortality and root browning in *Ocimum basilicum* grown with or without *Glomus mosseae* and/or *Fusarium oxysporum* f.sp. *basilici*

Treatment[a]	AM colonization (%)[b]	Plant mortality (%)	Root browning (%)
NM	0	0	0
NM + P	0	0	0
AM	58b	0	0
AM + *Fob*	40a	20a	6a
NM + *Fob*	0	33b	19b

[a]NM, non-mycorrhizal plants; P, phosphorus-treated plants; AM, mycorrhizal plants inoculated with *G. mosseae*; *Fob*, plants treated with *F. oxysporum* f.sp. *basilici*.
[b]Values represent means (n = 5) and for each parameter different letters within columns represent significant differences according to Tukey's test ($P \leq 0·05$).

Table 2 Leaf rosmarinic (RA) and caffeic acid (CA) concentrations, leaf phenolic concentration and antioxidant activity in *Ocimum basilicum* grown with or without *Glomus mosseae* and/or *Fusarium oxysporum* f.sp. *basilici*

Treatment[a]	Leaf RA (mg g^{-1} DW)[b]	Leaf CA (mg g^{-1} DW)	Leaf phenolic concentration (mg CA g^{-1} DW)	Leaf antioxidant activity (mg Trolox g^{-1} DW)
NM	16·8c	0·52a	69·48b	111·60b
NM + P	15·2bc	0·50a	55·22a	81·46a
AM	15·7c	0·52a	60·56ab	89·86a
AM + *Fob*	11·8ab	0·75a	53·10a	80·25a
NM + *Fob*	11·0a	0·70a	41·26a	61·50a

[a]NM, non-mycorrhizal plants; P, phosphorus-treated plants; AM, mycorrhizal plants inoculated with *G. mosseae*; *Fob*, plants treated with *F. oxysporum* f.sp. *basilici.*
[b]Values represent means (n = 5). Different letters within columns represent significant differences according to Tukey's test ($P \leq 0{\cdot}05$); DW, dry weight.

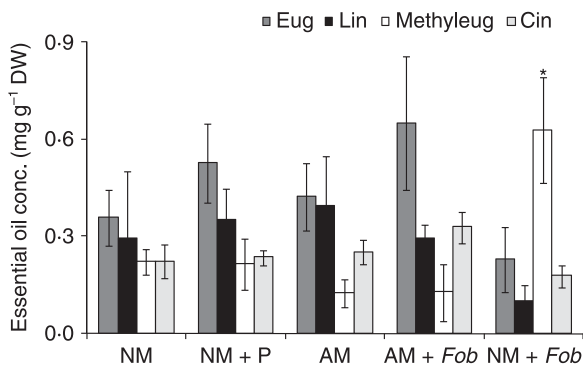

Figure 2 Essential oil compound concentrations (mg g^{-1} DW) in the leaves of *Ocimum basilicum* grown under various treatments. Eug, eugenol; Lin, linalool; Methyleug, methyleugenol; and Cin, 1,8-cineole. Means (n = 3–5) and SE bars are shown. An asterisk indicates a significant difference in methyleugenol concentration between the different treatments according to Tukey's test ($P \leq 0{\cdot}05$). NM, non-mycorrhizal plants; *P*, phosphorus-treated plants; AM, mycorrhizal plants inoculated with *G. mosseae; Fob*, plants treated with *Fusarium oxysporum* f.sp. *basilici.*

Table 3 Root rosmarinic (RA) and caffeic acid (CA) concentrations in *Ocimum basilicum* grown with or without *Glomus mosseae* and/or *Fusarium oxysporum* f.sp. *basilici*

Treatment[a]	Root RA (mg g^{-1} DW)[b]	Root CA (mg g^{-1} DW)
NM	24·7a	0·36ab
NM + P	34·3b	0·39bc
AM	37·8b	0·33a
AM + *Fob*	27·6a	0·44c
NM + *Fob*	23·9a	0·40bc

[a]NM, non-mycorrhizal plants; P, phosphorus-treated plants; AM, mycorrhizal plants inoculated with *G. mosseae*; *Fob*, plants treated with *F. oxysporum* f.sp. *basilici.*
[b]Values represent means (n = 10–15). Different letters within columns represent significant differences according to Tukey's test ($P \leq 0{\cdot}05$); DW, dry weight.

than most other treatments, except NM plants (Table 3). AM + *Fob* plants had significantly (P = 0·001) higher root CA concentrations than both NM and AM plants.

The most abundant essential oil compounds found in the leaves were eugenol (0·2–0·6 mg g^{-1} DW), linalool (0·1–0·4 mg g^{-1} DW), methyleugenol (0·1–0·6 mg g^{-1} DW) and 1,8-cineole (0·2–0·3 mg g^{-1} DW). The patterns of essential oil concentration were quite similar in NM, NM + P, AM and AM + *Fob* plants (Fig. 2). However, NM + *Fob* plants showed a different pattern. In these plants, methyleugenol concentration was much higher than that of eugenol. Methyleugenol concentration in NM + *Fob* plants was also significantly higher ($P \leq 0{\cdot}001$) than in all other treatments. Concentrations of other compounds did not vary significantly between treatments.

Discussion

The data presented here clearly show that colonization of basil roots by *G. mosseae* provides a certain level of bioprotection against *Fob* by reducing the damage caused by the pathogen (i.e. lower plant mortality and reduced root decay). This protective effect was not caused by enhanced P uptake in AM plants, as NM and AM plants treated with *Fob* had similar shoot P concentrations. As reviewed by Harrier & Watson (2004), improved mineral nutrition (e.g. P) does not always explain the bioprotective effect conferred by AMF, in agreement with the observations here. Furthermore, the results of the present study are fairly consistent with those of Liu *et al.* (2007) who demonstrated that *Medicago truncatula* colonized by *G. intraradices* showed a significant resistance to the bacterial pathogen *Xanthomonas campestris*. This resistance was not associated with an improved P uptake, as AM and NM plants were matched for P concentrations.

Interestingly, the bioprotective effect conferred by *G. mosseae* was not correlated with higher concentrations of phytochemicals (RA, CA or essential oil compounds). In contrast to the work of Toussaint *et al.* (2007), AM plants did not have higher shoot RA and CA concentrations. The discrepancy between the results obtained here and those of Toussaint *et al.* (2007) was possibly caused by differences in the type of fungal inoculum used (and inoculum strain), growing conditions, nutrient solution used in the experiments, or the age of the plants at harvest.

Furthermore, contrary to expectations, leaf RA concentrations were reduced by *Fob*, and AM + *Fob* plants did not have higher leaf RA concentrations than AM or NM + *Fob* plants. In fact, *Fob* inoculation was associated with reductions in leaf RA concentrations. On the other hand, the concentration of total phenolics and leaf antioxidant activity followed a similar trend to that of leaf RA concentrations. These findings suggest that under the current experimental conditions, RA could have been the predominant phenolic compound conferring antioxidant activity in the leaves of basil, as proposed by other authors (Juliani & Simon, 2002; Jayasinghe *et al*., 2003).

It has been speculated that RA can serve as a defence compound and feeding deterrent against herbivores, mainly in roots (Bais *et al*., 2002). However, in the present experiment, *Fob* did not increase root RA concentrations, but P supply (NM + P) and colonization with *G. mosseae* (AM) did. As NM + P and AM plants had the highest P uptake (see P content of shoots), it is therefore possible that root RA concentrations are affected by P uptake. This hypothesis would be in accordance with the data reported by Toussaint *et al.* (2007) in which RA concentration of NM roots was significantly correlated with root P content. Furthermore, as AM + *Fob* and NM + *Fob* had similar root CA concentrations, it was not possible to conclude that either RA or CA concentrations were correlated with the bioprotection effect conferred by *G. mosseae* against *Fob*.

In most treatments eugenol and linalool were the most abundant essential oil compounds in basil, confirming the data of Copetta *et al.* (2006). Eugenol is considered to be an antibacterial and antifungal agent (Gang *et al*., 2001), and is converted to methyleugenol by an *O*-methyltransferase. In the present study, NM + *Fob* plants had reduced concentrations of eugenol, but enhanced concentrations of methyleugenol, suggesting that under the sole influence of the pathogen there was an increased conversion of eugenol into methyleugenol compared to other treatments. This result is consistent with reports that showed an induction of *O*-methyltransferases as a defence response to the presence of fungal elicitors (Lewinsohn *et al*., 2000). The latter results are also consistent with the findings of Miele *et al.* (2001) that basil plants with reduced growth had higher levels of methyleugenol, similar to what was observed in the present study in plants treated with *Fob* only. As the presence of *Fob* caused growth reductions, it is therefore likely that the pathogen triggered the production of methyleugenol via increased activity of *O*-methyltransferase.

It has been suggested that the intake of considerable doses of methyleugenol (e.g. through consumption of fresh basil or pesto) is harmful (Miele *et al*., 2001). The chemical structure of methyleugenol is very close to that of estragole, a known genotoxic carcinogen (Gang *et al*., 2001; Miele *et al*., 2001). In this context, the lower concentrations of methyleugenol observed in the leaves of AM + *Fob* plants compared to NM + *Fob* plants are of significant importance. Indeed, the results suggest that the presence of *G. mosseae* prevents the accumulation of methyleugenol in *Fob*-infected plants. This result is somewhat consistent with that of Copetta *et al.* (2007), who observed slight reductions (although not significant) in methyleugenol concentrations in basil plants colonized by the same strain of *G. mosseae* (BEG 12, Biorize) compared to NM plants. Thus, colonization of basil with AMF could be used as a tool to reduce the accumulation of a potentially harmful compound in *Fob*-infected plants.

The data obtained also indicated that increasing P supply to NM plants does not significantly affect their growth, despite higher P uptake. Similar results were reported by Khaosaad *et al.* (2006), where two different varieties of NM *Origanum vulgare* had similar shoot biomass when supplied or not with P. In the present study AM plants grew better than NM + P plants, even though both took up similar amounts of P in their shoots (see P content of shoots). AM plants had similar shoot P concentration to NM and NM + P plants, suggesting that a factor other than P was limiting the growth of NM plants. Furthermore, AM, NM and NM + P plants also had similar leaf RA and CA concentrations and a similar accumulation pattern of essential oil compounds, regardless of P nutrition. These results are consistent with previous observations that improved P nutrition does not necessarily contribute to higher production of phytochemicals in basil leaves (Copetta *et al*., 2006; Toussaint *et al*., 2007).

Although the interaction between *G. mosseae* and *Fob* did not result in higher concentrations of phytochemicals (RA, CA or essential oil compounds) than in NM plants, it was demonstrated that this AM fungus can (i) promote the growth of basil; (ii) confer a protective effect against *Fob*; (iii) yield similar leaf RA and CA concentrations as NM plants under high P supply, consistent with previous results (Toussaint *et al*., 2007); and (iv) reduce the concentration of the harmful essential oil methyleugenol in *Fob*-infected basil plants. The protective effect of *G. mosseae* against *Fob* could not be associated with an increase in P content, leaf or root RA and CA concentrations nor an increase in essential oil compound concentrations.

Several mechanisms have been proposed to explain the bioprotective effect of AMF (Pozo & Azcón-Aguilar, 2007). One of these is the competition for colonization sites by beneficial and harmful fungi. In the present study, *Fob* was applied after the plants were well colonized by *G. mosseae*, which might have conferred a competitive advantage. Ultimately, the triggering of defence-related metabolites (other than the one investigated here) remains one of the principal hypotheses explaining the bioprotective effect conferred by AMF (St-Arnaud & Vujanovic, 2007). Therefore, combining novel analytical approaches with classical physiological techniques (Toussaint, 2007), will help understand the mechanisms underlying the bioprotective effect conferred by AMF to their host plants.

Finally, a possible way to reduce the infection caused by *Fob* in field-grown basil might be the treatment of the growing substrate with an AM inoculum 1–2 weeks prior to or at sowing. Such a method has some practical limitations, but there have been reports of successful inoculation in field experiments, as reviewed by Gosling

et al. (2006). Further field-based studies with seed- and soilborne inocula of *Fob* are needed to clarify the potential and the competitive advantage of this pre-treatment with AMF. Hence, appropriate AM inoculation methods, along with other favourable biocontrol and management practices might help reduce the incidence of *Fob* in the field.

Acknowledgements

This work was partially funded (MN and HV) by the Austrian Science Fund (FWF; project L194-B06). JPT is grateful to the University of Adelaide for both an International Postgraduate Research Scholarship and a Research Abroad Scholarship, which enabled participation in this project.

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