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Interactions between *Plasmopara helianthi, Glomus mosseae* and two plant activators in sunflower plants

Laura Tosi and Antonio Zazzerini
Dipartimento di Arboricoltura e Protezione delle Piante, Università degli Studi di Perugia, Borgo XX Giugno, 74, 06121 Perugia, Italy (Fax: +39755856482; E-mail: patveg@unipg.it)

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Abstract

Interactions between *Plasmopara helianthi, Glomus mosseae* and two plant activators DL-β-amino-n-butyric acid (BABA) and CGA 245704 (acibenzolar-S-methyl (BTH)) in sunflower plants susceptible to downy mildew were studied in four experiments using different methods of treatment and pathogen inoculation. Both chemicals were applied as soil drenches and foliar sprays, whereas *P. helianthi* infection was obtained by root and cotyledon inoculations of the seedlings. Soil drenches at the rates of 50 and 100 mg kg^{-1} soil of BABA and BTH given 1 and 3 days before *P. helianthi* inoculation, respectively to mycorrhizal plants, provided moderate protection against the pathogen (about 50–55%). Morphological changes and decrease in mycorrhizal colonization in roots of BTH-treated plants and in BTH-treated mycorrhizal plants were also observed. Delay in the emergence and reduction of the root systems were more evident at the highest concentration but decreased with time. These effects were absent with the BABA treatment.

Foliar spray treatment of BABA and BTH, applied at 4000 and 200 μg ml^{-1}, respectively (1 day post-inoculation) to mycorrhizal plants provided good protection (about 80%) against *P. helianthi* foliar infections. No effects on mycorrhizal colonization or on root systems were observed.

In vitro tests on the effect of the compounds on the mycorrhizal fungus showed that the germination of *G. mosseae* sporocarps increased with BABA treatment whereas it was greatly inhibited by BTH treatment.

Introduction

During the last few years, limitations in pesticide use due to toxicological and environmental problems and the increasing risk of fungicide resistance development have revalued biological and other control measures in plant protection. Many papers have reported the protective effects of bacteria, fungi and arbuscular mycorrhizae against plant pathogens and have discussed the different mechanisms that could contribute to the protective activity and interactions between these biological agents and pathogens (Weller, 1988; Upadhyay and Bharat, 1988; Adams, 1990; Hooker et al., 1994; Barea and Jeffries, 1995; Thomashow and Weller, 1995; Azcon-Aguilar and Barea, 1996).

Plants possess a wide variety of active defence mechanisms which affect and reduce disease progression. One of these inducible systemic responses, referred to as systemic acquired resistance (SAR), can be induced by biotic and abiotic agents. Also, the recent development of chemical plant activators such as salicylic acid (SA), 2,6 dichloroisonicotinic acid (INA), DL-β-amino-*n*-butyric acid (BABA) and acibenzolar-S-methyl (BTH) which are able to activate the SAR response in plants may represent important opportunities in plant disease control (Kessmann et al., 1994; Schneider et al., 1996; Sticher et al., 1997).

Most literature reports that the combination of arbuscular mycorrhizal (AM) fungi with biocontrol agents and/or plant-growth-promoting rhizosphere (PGPR) organisms is compatible and often determines an increase in bacterial and fungal populations and in mycorrhizal infection (Datnoff et al., 1995; Calvet et al., 1993; Nemec, 1994; 1997).

Furthermore, compatibility of biological control agents with agrochemicals is well documented (Kurle and Pfleger, 1994; Quarles, 1999) and represents another possibility for limiting the input of pesticides and a tool that will be utilized for crop protection strategies in IPM systems. On the other hand, no research has been carried out on the compatibility between arbuscular mycorrhizae and the application of plant activators for controlling plant disease.

Therefore the effect of two plant activators and mycorrhizae was studied on sunflower plants susceptible to *P. helianthi* Novot. The causal agent of downy mildew is one of the major diseases in sunflower cultivation causing severe yield losses. Although *P. helianthi* can be controlled by systemic fungicide treatment of seeds and the use of resistant varieties, many reports show its world-wide presence and an increase of its physiological races. Moreover, our previous works showed that this model 'sunflower–*P. helianthi*' was very suitable for studying the interactions between the pathogen, mycorrhizae (Tosi et al., 1993) and the effects of two plant activators BABA and CGA 245704 BTH (Tosi et al., 1998; 1999). So, the effects of these compounds were studied: applied as a soil drench or foliar spray, together with *G. mosseae* for protecting sunflower against downy mildew.

Materials and methods

Soil

The experimental soil was a sandy loam from Panicarola (PG), with 55 ppm Olsen P and pH 6.8. It was autoclaved at 121 °C for 2 h to eliminate naturally occurring endophytes.

Plant

All the experiments were carried out with susceptible sunflower plants (*Helianthus annuus* L., cv. Ala). Sunflower seeds were sown (two seeds/pot) in 8 cm diameter plastic pots containing 250 g of the experimental soil.

Mycorrhizal inoculum

The mycorrhizal fungus used was *Glomus mosseae* (Nicol. *et* Gerd.) Gedermann *et* Trappe (BEG 12), produced on *Trifolium pratense* L., var. Rajah stock plants. Inoculum, consisting of infected soil containing sporocarps, spores and infected root fragments and extramatrical mycelium, was placed in a layer, 2 cm below the seeds. In all experiments the amount of mycorrhizal inoculum was 300 g Kg^{-1}.

Pathogen inoculum

The isolate of *Plasmopora helianthi* (race 1 or 'European race') was maintained on susceptible sunflower plants (cv. Ala) by inoculating pre-germinated seeds and growing them in pots containing perlite in a separate growth chamber at an alternate day/night temperature 20/18 ± 2 °C, 60–70% day/night relative humidity with 12 h of light (180 $\mu Em^{-2} s^{-1}$) per day. After 2 weeks sporulation was induced by covering the infected plants with plastic bags for 48 h to provide a saturated atmosphere (Cohen and Sackston, 1973). Zoosporangia (and zoosporangiophores) were gently brushed from the cotyledons and leaves into distilled water; their concentration was determined with a haemocytometer and adjusted as necessary with distilled water.

Four different experiments were performed using different methods of treatment and pathogen inoculation. In the first two experiments, sunflower seeds (two seeds per pot) were sown in 8 cm-diameter pots containing 250 g of experimental soil. The root inoculation of seedlings was obtained by distributing the zoosporangia suspension (700 zoosporangia per g soil) over the soil surface (20 ml per pot) 7 days after sowing. In the last two experiments, 20 plants for cotyledons inoculation were grown in the same experimental soil in pots (30 cm × 21 cm × 6 cm) in a greenhouse at 20 °C. Foliar inoculation of the experimental plants was obtained by spraying the sporangial suspension (10^6 zoosporangia per ml) on cotyledons, a week after sowing. Inoculated seedlings were covered with plastic bags and kept at 18 °C for 24 h, and then uncovered and transferred to a greenhouse at controlled conditions.

Plant activators and application

BABA and CGA 245704 (acibenzolar-S-methyl, or benzothiadiazole) (BTH) were used as chemical

inducers and were obtained from Sigma as powder (minimum 95%) and from Novartis, as 50% water dispersible granule (BION WG 50), respectively. Both chemicals were applied as soil drenches (30 ml per pot) and as a foliar spray to incipient rainoff.

In the first experiment, BABA was applied at 50 and 100 mg kg^{-1} soil 1 day before inoculation with *P. helianthi* (Tosi et al., 1998). Each treatment was replicated six times. The second experiment was conducted in the same way as experiment 1, but using BTH applied at the same rates 3 days before inoculation with the pathogen (Tosi et al., 1999). In the third experiment, sunflower plant cotyledons were uniformely sprayed with a zoosporangia suspension of *P. helianthi* and BABA was applied as a foliar spray treatment (2000 and 4000 μg ml^{-1}) 1 day post-inoculation. The fourth experiment was conducted in the same way as experiment 3, but BTH was tested at 100 and 200 μg ml^{-1}.

Plants from all experiments were fertilized once per week with a Hoagland's solution lacking phosphorus and grown in a greenhouse at an alternate day/night temperature of 20/18 ± 2 °C, 60–70% day/night relative humidity with 12 h of daylight (180 μEm^{-2} s^{-1}) for 20 days. All experiments were repeated twice.

Evaluation of the degree of infection of the two endophytes

Six plants from all experiments were cleared, stained and examined for mycorrhizal and pathogen infection (Giovannetti et al., 1991). Segments of roots were selected under the dissecting microscope, mounted on slides and examined under a light microscope for monitoring the development of infection by the endophyte and the pathogen.

Evaluation of systemic infection by P. helianthi

At the end of each experiment (13 days after inoculation), plants were placed for 48 h in a saturated atmosphere to assess fungal sporulation on hypocotyls, cotyledons and true leaves. Twenty plants for each treatment were removed from soil and sporulation of *P. helianthi* was assessed visually with or without a dissecting microscope. All hypocotyls that seemed to be healthy were cut into sections 2–3 cm long and were incubated on moist filter paper in Petri dishes at 20 °C for 24–48 h in the dark to induce sporulation. Percentage of infected plants was determined by counting plants with systemic infection (showing chlorosis, stunting and sporulation on cotyledons and true leaves) and plants with sporulation on hypocotyls.

Evaluation of the effects of plant activators on germination and mycelial growth of the arbuscular mycorrhizal fungus

This experiment investigated the influence of BABA and BTH on germination and mycelial growth of *G. mosseae*, used as a non-target soil microorganism. Ten sporocarps of *G. mosseae* were sandwiched between two Millipore membranes (0.45 μm diameter pores). The sandwiches were transferred to Petri dishes containing sterile grit moistened with BABA and BTH at concentrations of 0, 50, 100 and 200 μg ml^{-1}, and incubated in the dark at 24 °C. After 10 days, the sandwiches were removed from the grit, carefully opened and the sporocarps were stained with 0.05% trypan blue in lactic acid. Hyphal growth was assessed with a Wild dissecting microscope at 50 magnification, using the gridline intersect method (grid 47 mm diam, lines 3.66 mm apart) (Giovannetti and Mosse, 1980). Ten replicate sandwiches, containing 10 sporocarps each, were assessed for each treatment.

Statistical analysis

Percentages of infected plants were analysed by analysis of variance (Anova) and significant differences were detected using Fisher's Protected LSD test. The percentage protection was calculated as $100\,(1-x/y)$, where x and y were the percentage of infected plants in treated-infected and untreated-infected plants, respectively.

Effects of plant activators on the percentage of germinated sporocarps of *G. mosseae* were analysed by Anova; data on mycelial growth of germinated sporocarps were compared using Student's *t*-test (standard errors are reported in Table 4).

Arcsine transformation was used for all precentage data.

Standard errors (SE) and significant differences (Student's *t*-test) between infection precentages of infected and infected mycorrhizal plants for each treatment of experiment 2 (BTH at 100 mg kg^{-1} soil) are reported in Figure 1.

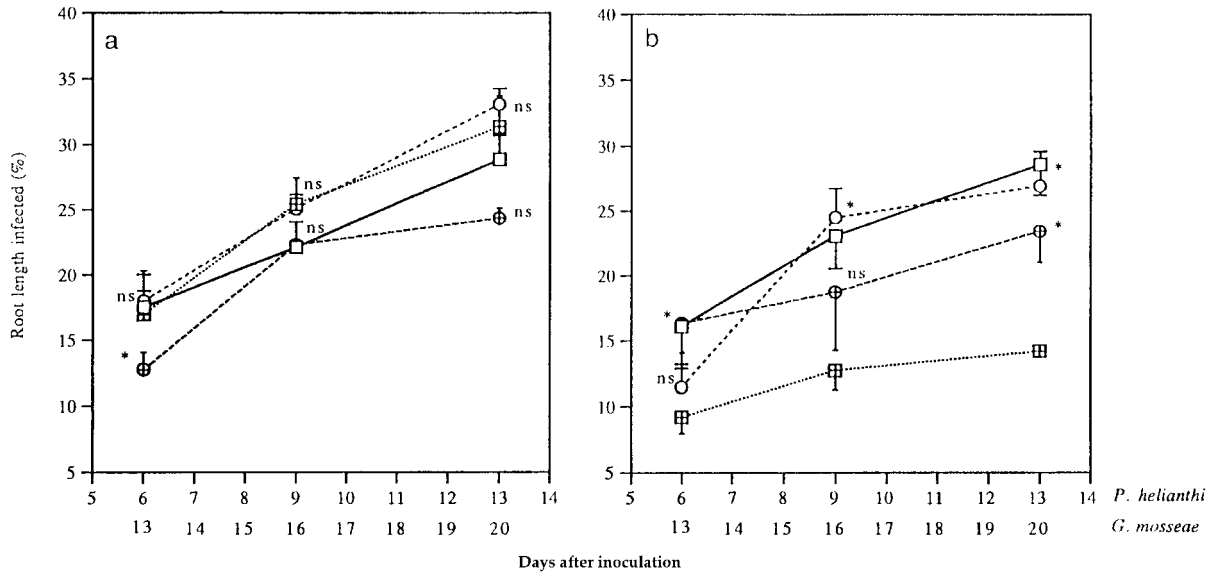

Figure 1. Experiment 2-Percentage root length infected by *P. helianthi* in infected plants (——□——), by *G. mosseae* in mycorrhizal plants (. . . . . ⊞. . . . .) and by *G. mosseae* (– – –◯– – –) and by *P. helianthi* (– – –⊕– – –) in mycorrhizal infected plants (a). Percentage root infected by *P. helianthi* in BTH-treated infected plants (——□——), by *G. mosseae* in BTH-treated mycorrhizal plants (. . . . . ⊞. . . . .) and by *G. mosseae* (– – –◯– – –) and by *P. helianthi* (– – –⊕– – –) in BTH-treated mycorrhizal infected plants (b) at different days after *G. mosseae* and/or *P. helianthi* inoculation; BTH was applied as soil drench at 100 mg kg^{-1} soil. *t*-test: ns, not significant; *significant at $P \leq 0.05$ (for further details see text). Vertical bars represent ±SE.

Results

Results from the first two experiments are reported in Table 1. Both plant activators, when applied at lower rate, provided moderate protection against *P. helianthi*, as previously reported (Tosi et al., 1998;1999). Infected plants treated with BABA and BTH showed 51% and 66% protection at 100 mg kg^{-1} soil, respectively. Macroscopic and microscopic observations during the first two experiments revealed a reduction of root systems and of the degree of colonization by the mycorrhizal endophyte following BABA and BTH treatments; these effects however decreased with time.

Degree of infection by the two endophytes

At the beginning of the first experiment (inoculation of the pathogen) mycorrhizal infection was 14% and increased up to 37% by the end of the experiment (13 days). In mycorrhizal plants infected with *P. helianthi*, the colonization of roots by the endophyte was similar to mycorrhizal plants, whereas *P. helianthi* colonized the roots slowly reaching about 23% at the 13th day. Infection by the pathogen increased from 19% at the 6th day up to 38% at the end of the experiment, whereas in BABA-treated infected plants (50 mg kg^{-1} soil) the pathogen occurred in about 29% of the root length at the 13th day.

In BABA-treated mycorrhizal plants the colonization of *G. mosseae* was very low 6 days after the beginning of the experiment (12%), but mycorrhizal infection increased reaching 29% at the 13th day. In BABA-treated mycorrhizal infected plants, root colonization by the endophyte initially appeared slightly higher (17%) than that of BABA-treated mycorrhizal plants, but at the end of the experiment *G. mosseae* occurred in about 25% of the root length. Root length infected by *P. helianthi* was significantly lower (17%) at the end of the experiment.

Results of the degree of infection by the two endophytes were similar when BABA was applied at 100 mg kg^{-1} soil.

Degree of infection by the two endophytes

In the second experiment with BTH, applied at both rates, the effects of the compound on mycorrhizal infection were more marked compared to those

Table 1. Effect of BABA and BTH, applied as soil drench at 50 and 100 mg Kg^{-1} soil, on mycorrhizal infection (*G. mosseae* 300 g Kg^{-1} soil) on *P. helianthi* root infection of sunflower plants (cv. Ala)

Treatments	Mycorrhizal infection* (%)	Infected plants* (%)	Protection (%)	Treatments	Mycorrhizal infection* (%)	Infected plants* (%)	Protection (%)
Infected control plants	—	80 b	—	Infected control plants	—	83 c	—
Mycorrhizal plants	37 c	—	—	Mycorrhizal plants	34 cd	—	—
Mycorrhizal infected plants	37 c	39 a	51	Mycorrhizal infected plants	35 d	39 ab	53
BABA-treated infected plants (50 mg)	—	50 a	37	BTH-treated infected plants (50 mg)	—	55 bc	34
BABA-treated infected plants (100 mg)	—	39 a	51	BTH-treated infected plants (100 mg)	—	28 ab	66
BABA-treated mycorrhizal plants (50 mg)	30 b	—	—	BTH-treated mycorrhizal plants (50 mg)	24 b	—	—
BABA-treated mycorrhizal plants (100 mg)	29 b	—	—	BTH-treated mycorrhizal plants (100 mg)	14 a	—	—
BABA-treated mycorrhizal infected plants (50 mg)	25 a	44 a	45	BTH-treated mycorrhizal infected plants (50 mg)	33 cd	44 abc	47
BABA-treated mycorrhizal infected plants (100mg)	24 a	39 a	51	BTH-treated mycorrhizal infected plants (100 mg)	27 bc	28 a	66

*The percentage root length by *G. mossae* and the percentage of infected plants were determined 20 days after inoculation with *G. mosseae* and 13 days after *P. helianthi* inoculation, respectively. Mean percentages in each column were compared using Anova. Values followed by the same letter within a column are not significantly different at $P \leq 0.05$ according to Fisher's Protected LSD test.

obtained with BABA. Mycorrhizal infection increased from 17% at the 6th day up to 33% at the end of the experiment. In mycorrhizal infected plants, root colonization by the mycorrhizal fungus increased in the same way as in mycorrhizal plants, up to 34% at the 13th day; root infection by the pathogen occurred in only 27% of the root length at the end of the experiment (Figure 1).

Infection by *P. helianthi* was about 17% at the 6th day and reached 31% at the 13th day; in BTH-treated infected plants (at both rates) *P. helianthi* occurred in about 29% of the root length (13th day).

In BTH-treated mycorrhizal plants, at the lower rate, the mycorrhizal endophyte colonized the roots slowly, being 15% and 24%, respectively 6 and 13 days after the beginning of the experiment compared to mycorrhizal plants. In BTH-treated mycorrhizal plants, at the higher rate, mycorrhizal colonization reached only 14% of the root length at the end of the experiment. In BTH-treated mycorrhizal infected plants (50 mg kg^{-1} soil), mycorrhizal infection was initially low, but significantly increased with time so that the percentage root length (33%) was higher than in BTH-treated mycorrhizal plants. Also, *P. helianthi* infection was lower (about 27%) 13 days after inoculation compared with BTH-treated infected plants (31%). A similar behaviour on the degree of infection by both endophytes was observed in BTH-treated mycorrhizal infected plants at the higher rate (Figure 1).

Microscopical examinations

In the first two experiments microscopical examinations of root segments from all treatments indicated that the typical structures of the pathogen and the mycorrhizal endophyte (intracellular haustoria and arbuscules respectively) were clearly visible 6 days after inoculation of the pathogen, whereas oospores of the pathogen appeared after 9 days. Generally, in roots of plants (mycorrhizal and/or infected) treated with BTH, at both rates, the structures of both endophythes occurred at the same time even if they were present in a small number with respect to controls (Figure 2).

Plant growth parameters

In the second experiment, a reduction in root system length of BTH-treated plants and in BTH-treated mycorrhizal plants was also observed. At the beginning of the experiment with BTH applied at 50 mg kg^{-1} soil, the root systems of BTH-treated plants were reduced by 52% with respect to control plants and the root systems of BTH-treated mycorrhizal plants by 41%

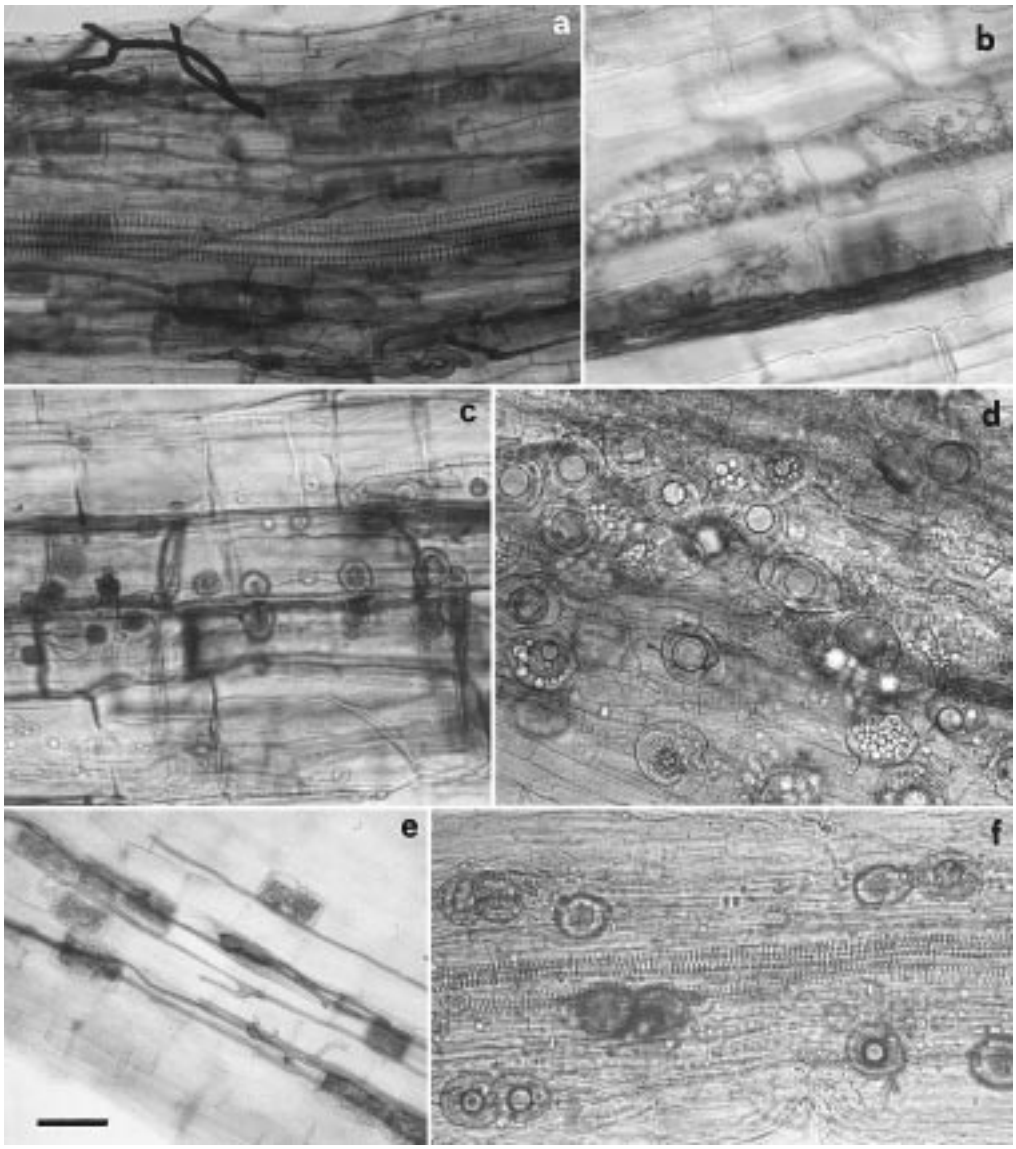

Figure 2. Light micrographs of hyphal structures of *P. helianthi* and *G. mosseae* in sunflower roots treated or not with BTH at 50 mg kg^{-1} soil. Sunflower root colonized by *G. mosseae* 16 days after inoculation (a). Arbuscules formed by AM fungus in some root cells (b). Intercellular hyphae and haustoria in root cells invaded by *P. helianthi* 9 days after inoculation (c). Root completely filled with oospores of *P. helianthi* 13 days after inoculation (d). BTH-treated mycorrhizal root poorly infected by *G. mosseae* 16 days after inoculation (e). BTH-treated infected root showing a small number of oospores of *P. helianthi* (f) (scale bars = 25 μm (b and c); 50 μm (a and d–f)).

with respect to mycorrhizal plants (Table 2). These effects, more evident when BTH was applied at the highest concentration (reduction of 60% and 66% respectively) (Table 2), decreased with time. Moreover, BTH-treated plants showed a slight delay in the emergence compared to control plants. Microscopical examinations of BTH-treated roots showed a delay in the formation of apical and lateral roots, probably due to a reduced mitotic activity. These negative effects were almost absent in BABA treatments (data not shown).

Results from the last two experiments (Table 3) showed that both plant activators were effective in protecting sunflower plants against *P. helianthi* foliar

Table 2. Total lenth* (cm) of roots in non-mycorrhizal (*G. mosseae* 300 g Kg^{-1} soil) plants, in untreated and treated plants with BTH (50 and 100 mg Kg^{-1} soil) with or without *P. helianthi* infection

Treatments	Harvest days (after *P. helianthi* infection)							
	BTH 50 mg kg^{-1} soil				BTH 100 mg kg^{-1} soil			
	0	6	9	13	0	6	9	13
Control plants	142 (103–182) c	203 (186–221) de	275 (241–308) d	316 (274–352) cd	122 (90–149) c	214 (196–239) dc	292 (282–308) cd	328 (306–352) cd
Control infected plants	—	242 (186–330) f	233 (184–306) c	292 (268–314) c	—	265 (130–381) e	265 (215–304) c	277 (256–322) bc
Mycorrhizal plants	98 (78–116) b	231 (196–302) ef	247 (197–285) cd	355 (298–406) e	98 (58–130) b	247 (98–460) dc	338 (290–464) d	342 (218–420) d
Mycorrhizal-infected plants	—	172 (153–183) cd	243 (215–302) cd	343 (297–402) de	—	238 (192–295) dc	220 (158–354) ab	304 (212–398) cd
BTH-treated plants	83 (64–105) b	123 (97–152) a	153 (133–178) a	175 (140–207) a	41 (20–60) a	133 (73–220) abc	161 (134–201) a	154 (120–214) a
BTH-treated infected plants	—	134 (118–154) ab	162 (132–202) a	192 (174–212) ab	—	101 (68–152) a	158 (95–232) a	160 (124–212) a
BTH-treated mycorrhizal plants	47 (36–62) a	115 (98–132) a	164 (128–204) ab	213 (186–231) b	39 (19–58) a	109 (87–126) ab	166 (84–290) a	232 (168–328) b
BTH-treated mycorrhizal infected plants	—	157 (139–186) bc	195 (179–212) b	207 (193-220) b	—	131 (48–185) ab	189 (130–247) ab	237 (160–335) b

*Mean and range of six replicates. Mean in each column were compared using Anova. Values followed by the same letter within a column are not significantly at $P \leq 0.05$ according to Fisher's Protected LSD test.

Table 3. Effect of BABA (2000 and 4000 μ ml^{-1}) and BTH (100 and 200 μ ml^{-1}), applied 1 day post-inoculation as foliar spray treatment, on mycorrhizal infection (*G. mosseae* 300 g kg^{-1} soil) and on the cotyledons inoculations with *P. helianthi* in sunflower plants (cv Ala)

Treatments	Mycorrhizal infection* (%)	Infected plants* (%)	Protection (%)	Treatments	Mycorrhizal infection* (%)	Infected plants* (%)	Protection (%)
Infected control plants	—	71 c	—	Infected control plants	—	79 d	—
Mycorrhizal plants	36 b	—	—	Mycorrhizal plants	34 b	—	—
Mycorrhizal infected plants	35 b	69 c	3	Mycorrhizal infected plants	33 ab	76 d	4
BABA-treated infected plants (2000 μg ml^{-1})	—	22 b	69	BTH-treated infected plants (100 μg ml^{-1})	—	26 bc	67
BABA-treated infected plants (4000 μg ml^{-1})	—	15 a	79	BTH-treated infected plants (200 μg ml^{-1})	—	10 a	87
BABA-treated mycorrhizal plants (2000 μg ml^{-1})	33 a	—	—	BTH-treated mycorrhizal plants (100 μg ml^{-1})	33 ab	—	—
BABA-treated mycorrhizal plants (4000 μg ml^{-1})	34 ab	—	—	BTH-treated mycorrhizal plants (200 μg ml^{-1})	34 b	—	—
BABA-treated mycorrhizal infected plants (2000 μg ml^{-1})	33 a	19 ab	73	BTH-treated mycorrhizal infected plants (100 μg ml^{-1})	32 a	38 c	52
BABA-treated mycorrhizal infected plants (4000 μg ml^{-1})	34 ab	14 a	80	BTH-treated mycorrhizal infected plants (200 μg ml^{-1})	34 ab	15 ab	81

*The percentage root length infected by *G. mosseae* and the percentage of infected plants were determined 21 days after inoculation with *G. mosseae* and 15 days after *P. helianthi* inoculation, respectively. Mean percentage in each column were compared using Anova. Values followed by the same letter within a column are not significantly different at $P \leq 0.05$ according to Fisher's Protected LSD test.

Table 4. Effect of BABA and BTH, at different concentrations, on germination and hyphal growth of *G. mosseae* sporocarps in the 'sandwich system'

Concentration (μg ml^{-1})	BABA		BTH	
	Germinated sporocarps[a] (%)	Hyphal length per germinated sporocarp[b] (cm)	Germinated sporocarps[a] (%)	Hyphal length per germinated sporocarp[b] (cm)
0 (control)	37 (20–50) a	6.84 ± 1.01	60 (40–90) a	11.74 ± 0.69
50	46 (0–70) ab	3.62 ± 0.99 ns	26 (10–40) b	7.06 ± 1.30*
100	74 (50–90) bc	5.92 ± 0.06 ns	20 (0–40) b	6.8 ± 1.57*
200	77 (40–100) c	8.96 ± 0.01 ns	4 (0–10) c	16.65 ± 1.05*

[a]Mean and range of five replications. Mean percentage in each column were compared using Anova. Values followed by the same letter within a column are not significantly different at $P \leq 0.05$ according to Fisher's Protected LSD test.
[b]Mean and standard error of five replicates (10 sporocarps per each trail). The asterix indicates significant differences betweeen treatments and control: *significant at $P \leq 0.05$; ns, not significant

infection. Both compounds, at the highest rates, either when applied alone or in combination with the mycorrhizal fungus provided good protection (about 80%). No reduction in root systems and/or in mycorrhizal colonization were observed with the two chemicals applied as foliar spray treatment. In particular, in BABA- and BTH-treated infected plants and also in BABA- and BTH-treated mycorrhizal infected plants

sporulation was lower than that in infected plants and mycorrhizal infected plants respectively. In these experiments, the mycorrhizal colonization increased rapidly in all treatments (data not shown) and reached about 34–35% of root length at the end of the experiment. However, root infection by *G. mosseae* alone did not reduce the severity of cotyledon inoculation of *P. helianthi* (Table 3).

Effects of plant activators on germination and mycelial growth of the arbuscular mycorrhizal fungus

In vitro tests showed that BTH treatment at the highest concentration (200 $\mu g\,ml^{-1}$), greatly inhibited sporocarp germination, whereas BABA treatment increased the percentage of germinated sporocarps (Table 4). It is interesting to note that at the highest rate of BTH treatment the mycelial length per germinated sporocarp was significantly greater than that of the control and the other two lowest BTH rates. No significant differences were observed on hyphal length/germinated sporocarp with BABA treatment at all rates tested.

Discussion

These results show that both plant activators (BABA and BTH), when applied in combination with an arbuscular mycorrhizal fungus (*G. mosseae*), provided protection against sunflower downy mildew *in vivo*. This study confirms that these compounds induce different levels of protection according to the methods of treatment (soil drench and foliar spray) and pathogen inoculation. When applied as soil drenches (1 and 3 days before root inoculation of the pathogen) these compounds gave moderate levels of protection (about 50–55%), whereas when applied as foliar spray, 1 day after cotyledon inoculation, they provided higher levels of control (about 80%) against *P. helianthi*.

With regard to the use of both plant activators, as soil drenches, in combination with *G. mosseae*, the evidence presented shows: (1) that when BTH was applied as a soil drench the compound produced a reduction of root systems and a slight delay in the emergence in treated and treated mycorrhizal plants; (2) the degree of root colonization by the mycorrhizal endophythe is lower than untreated mycorrhizal plants; (3) these negative effects on the root system and mycorrhizal infection decrease with time; (4) BABA treatment determines neither changes of root system nor mycorrhizal colonization.

In the last two experiments using *P. helianthi* foliar infection, a very protective effect of both compounds, applied as foliar spray, in combination with *G. mosseae* was observed. These results show: (1) a specific effect of both plant activators as inducers of resistance in sunflower against downy mildew than a protective effect of *G. mosseae*; (2) arbuscular mycorrhizal infection by *G. mosseae* alone does not substantially reduce *P. helianthi* foliar infection; (3) when both compounds are applied as foliar treatment no effects on root system and mycorrhizal colonization are observed. Moreover, the non-protective effect of *G. mosseae* against *P. helianthi* foliar infection should not be surprising because these data confirm previous observations suggesting that the reduced severity of root infection by *P. helianthi* in mycorrhizal plants could be attributed to a mechanism of infection-site and nutritional-site occupation (Tosi et al., 1993).

Both compounds exhibited direct activity *in vitro* on *G. mosseae* sporocarps, whereas they had no direct antimicrobial activity *in vitro* against *P. helianthi* (Tosi et al., 1998; 1999). Particularly, BTH treatment greatly inhibited the germination of *G. mosseae* sporocarps and it is interesting to note that at the highest concentration tested (200 $\mu g\,ml^{-1}$) the few germinated sporocarps seemed to be tolerant, because they showed a longer hyphal length per germinated sporocarp. On the contrary, sporocarp germination increased with BABA treatment, but hyphal length of germinated sporocarps did not significantly differ from control.

Chemical plant activators, when utilized against soilborne fungal pathogens may have effects on non-target soil and rhizosphere microorganisms, and may affect arbuscular mycorrhizal symbiosis. The evaluation of these agents should therefore include tests of their effects on mycorrhizal activity. This is particularly important in view of the management strategy of introducing new chemical compounds into agricultural systems, which do not affect the survival of non-target organisms such as arbuscular mycorrhizal fungi.

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