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RESEARCH ARTICLE

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Effect of the fungicide benomyl on spore germination and hyphal length of the arbuscular mycorrhizal fungus *Glomus mosseae*

Summary The fungicide benomyl inhibited spore germination and hyphal length of the arbuscular mycorrhizal fungus *Glomus mosseae* when applied at doses of 21.25 μg/ml (agronomic dose), 10.62 μg/ml and 10 μg/ml. *G. mosseae* was able to germinate in the presence of 2.12 μg/ml of benomyl, and the percentage of spore germination was unaffected by dosis of 0.1, 0.01 and 0.001 μg/ml of the fungicide. However, all doses of fungicide tested in this study decreased the hyphal length. When ungerminated *G. mosseae* spores previously exposed to benomyl were transferred to water-agar medium without benomyl, the maximum germination was 16%. Small spores of *G. mosseae* were more resistant to benomyl than the larger ones. Our results show some of the factors which can explain the variability of the effect of benomyl on arbuscular mycorrhizal fungi.

Key words *Glomus mosseae* · Arbuscular mycorrhizae · Benomyl · Spore germination · Fungicide

Introduction

Arbuscular mycorrhizal (AM) symbioses are essential components of most plant systems, and the beneficial role that AM fungi play in agricultural production is well known [2]. Although the use of pesticides is fundamental for soybean cultivation, there are not many studies about their effects on plant growth and on nontarget microorganisms such as AM fungi [15]. However, benomyl, like many other fungicides, has been reported to reduce AM fungi under some conditions. The effects of fungicides on AM symbiosis depend on environmental factors such as soil, crop system, climate, and also on the AM fungal strains themselves. This difficults the study of the effect of fungicides on AM fungus formation and function [16]. As AM fungi cannot be cultured axenically in the absence of the plant host, the spore germination test has been used to study the direct effect of pesticides on AM fungi [15].

Systemic fungicides can be applied to soil around plants and are absorbed by roots and translocated to other parts of the plant [11]. These kinds of fungicides are of great interest due to their persistence in the plant and their action on the AM fungi, either on their vegetative or on their resistance structures [12]. Benomyl is among the most frequently used systemic fungicides against pathogenic fungi of cereals and oleaginous plants [10]. It has been found to affect negatively AM symbioses by delaying or preventing the formation of AM symbiosis between fungi and roots and by decreasing plant P-uptake [8, 13, 19]. Benomyl suppresses mycelial growth by preventing nuclear division. In fact, this fungicide inhibits mitosis by blocking the formation of β-tubuline and microtubules when chromosomes are separated [6, 9, 11]. This work aims to find out some possible factors implicated in the sensitivity and resistance of spores of *Glomus mosseae* to benomyl.

Materials and methods

Sporocarps and spores were isolated by the wet-sieving technique [5] and identified as *Glomus mosseae* [4]. One hundred *G. mosseae* spores were inoculated to alfalfa plant pots. These plants were cultured for four months until their roots became well colonized (80% root length) and new *G. mosseae* spores were developed (41 spores/g of soil).

Influence of benomyl on spore germination The effect of the fungicide benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate, $C_{14}H_{18}N_4O_3$] from Dupont (Benlate, 50% active

ingredient) on the germination of *G. mosseae* spores was tested in vitro on 1% sterile water agar (Difco-Bacto). We added 10 mM 2-(N-morpholin)ethane sulfonic acid (MES) to 1% water agar to maintain the pH of the medium at 7 throughout the experiment. The fungicide was added to the agar of each Petri dish at the concentration of 21.25 μg/ml (equivalent to agronomic dose), 10.62, 2.12, 0.1, 0.01 and 0.001 μg/ml. Five to eight surface-sterilized spores were placed in each Petri dish [14]. Five replicates of each fungicide doses and controls were used. The plates were incubated at 25ºC and spore germination was checked under a light microscope after 6 days until 19 days of incubation. After 19 days hyphal length was also measured by using the gridline intersect method [7]. The diameters of *G. mosseae* spores treated with 0, 0.1 and 0.01 μg/ml. were measured under light microscope.

Sensitivity of spores to benomyl Ungerminated spores exposed to fungicides were washed with sterile water and transferred to freshly-prepared plates of water-agar without fungicides. These were incubated as above and the percentage of germination was checked after seven days.

Statistical analysis Data obtained were subjected to analysis of variance (ANOVA). Data obtained were arcsine-transformed before analysis. The means were compared by Tukey, Duncan and Scheffé multiple range tests at the 5% level.

Results and Discussion

Influence of benomyl on spore germination Table 1 shows that spore germination in the controls was 57%. The germination of *G. mosseae* spores was completely inhibited on media containing 10, 10.62 and 21.25 μg/ml of benomyl. In the presence of 1 and 2.12 μg/ml of benomyl the percentage of spore germination were 12 and 17% respectively. At concentrations of 0.1, 0.01 and 0.001 μg/ml of benomyl the percentage of spore germination increased to 64, 67 and 72%, respectively. ANOVA showed significant results between treatments and the Tukey, Duncan and Scheffé tests showed the same results.

Table 1 Effect of benomyl on the percentage of spore germination and hyphal length of the germ tube of *Glomus mosseae*

Amount of benomyl applied (μl/ml)	Percent spore germination*	Hyphal length* (mm)
0	57 (c)	74.28 (c)
0.001	72 (c)	12.95 (b)
0.01	68 (c)	12.66 (b)
0.1	64 (c)	12.15 (b)
1	17 (b)	0 (a)
2.12	12 (b)	0 (a)
10	0 (a)	0 (a)
10.62	0 (a)	0 (a)
21.25	0 (a)	0 (a)

*Column values followed by the same letter are not significant as Tukey, Duncan and Scheffé tests (P = 0.05).

The regression line between percentage of spore germination and benomyl concentration (C) showed the equation

$$y = 23.04 – 19 \log C$$

$$r = –0.9547, P = 0.0002$$

This equation indicates that benomyl concentrations equal or higher than 1 μg/ml decreased significantly the percentage of spore germination. Concentrations of benomyl equal to or lower than 0.1 μg/ml did not affect the percentage of spore germination. All doses of fungicide tested decreased significantly the length of the germ tube.

At 0.1 and 0.01 μg/ml benomyl concentrations, the largest spores (with a diameter of more than 40 μm) showed a lower rate of germination, than the small ones (21–30 and 31–40 μm diameter) (Table 2).

Table 2 Effect of benomyl on the percentage of germination of Glomus mosseae spores with different diameters

Diameter of spores (μm)	Concentration of benomyl applied (μl/ml)*		
	0	0.1	0.01
21–30	33.3 (c)	43.7 (c)	35.3 (c)
31–40	50.1 (c)	50.2 (c)	58.8 (c)
41–50	16.7 (b)	6.2 (a)	5.8 (a)

*Column values followed by the same letter are not significant as Tukey, Duncan and Scheffé tests (P = 0.05).

Sensitivity of spores to benomyl Spores exposed to 21.25 and 10.62 μg/ml of benomyl did not germinate when they were transferred to water-agar plates. The spores exposed to 10, 2.12 and 1 μg/ml of benomyl reached the percentage of 7, 11.7 and 16.6% germination, but they did not develop mycelia when they were transferred to agar-water medium (Table 3).

Table 3 Effect of benomyl on the percentage of germination and hyphal length of the germ tube of *Glomus mosseae* spores transferred to water-agar

Amount of benomyl applied (μl/ml)	Percent spore germination*	Hyphal length
1	16.6 (b)	0
2.12	11.7 (b)	0
10	7 (b)	0
10.62	0 (a)	0
21.25	0 (a)	0

*Column values followed by the same letter are not significant as Tukey, Duncan and Scheffé tests (P = 0.05).

The use of spores allows to study the direct effect of fungicides on AM fungi in the absence of plants. Our results showed that 0.1, 0.01 and 0.001 μg/ml of benomyl did not inhibit the germination of *G. mosseae* spores, but inhibited the hyphal length of the germ tube. The walls of the germ tube are more permeable than the multistratified walls of the spores. This suggests that the fungicide benomyl acted when the spore walls opened as the germ tube emerged from the spore. The germ tube is the most sensitive AM fungal structure [18]. The sensitivity of AM fungi to fungicides is variable [15]. In fact the effect of benomyl on *G. mosseae*

spores differs from that on *Glomus caledonicum* spores [3]. 10 ng/ml benomyl doses inhibit spore germination and hyphal length of *G. caledonicum*. However, our results showed that *G. mosseae* was able to germinate in the presence of 2.12 µg/ml of benomyl. Spore germination of *G. mosseae* was inhibited in the presence of 10 µg/ml of benomyl, but when these spores were transferred to Petri dish with agar-water without herbicide, the negative effect of benomyl decreased. However, this negative effect of benomyl on the hyphal length of the transferred spores was found even when the spores were exposed to the lowest dose of benomyl (1 µg/ml) used in our study. Nevertheless, the spores of *G. caledonicum* exposed to 0.1 and 10 µg of benomyl reached 95% germination and hyphal lengths similar to those of the controls when they were transferred to water-agar medium [3]. These results show that fungicidal or fungistatic effects of benomyl may vary in different AM fungi. Besides, our results also indicate that the sensitivity of AM fungi depends not only on fungal strains but also on the stage of fungal development.

The size of the spores is another factor to be taken into account in studies on the effect of fungicides on AM fungi. Our results show that smaller spores were more resistant than bigger ones to benomyl. This fact might be due to the modification of spore walls during their maturity allowing substances to enter the spores, which can affect and modify their metabolism [1, 17].

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