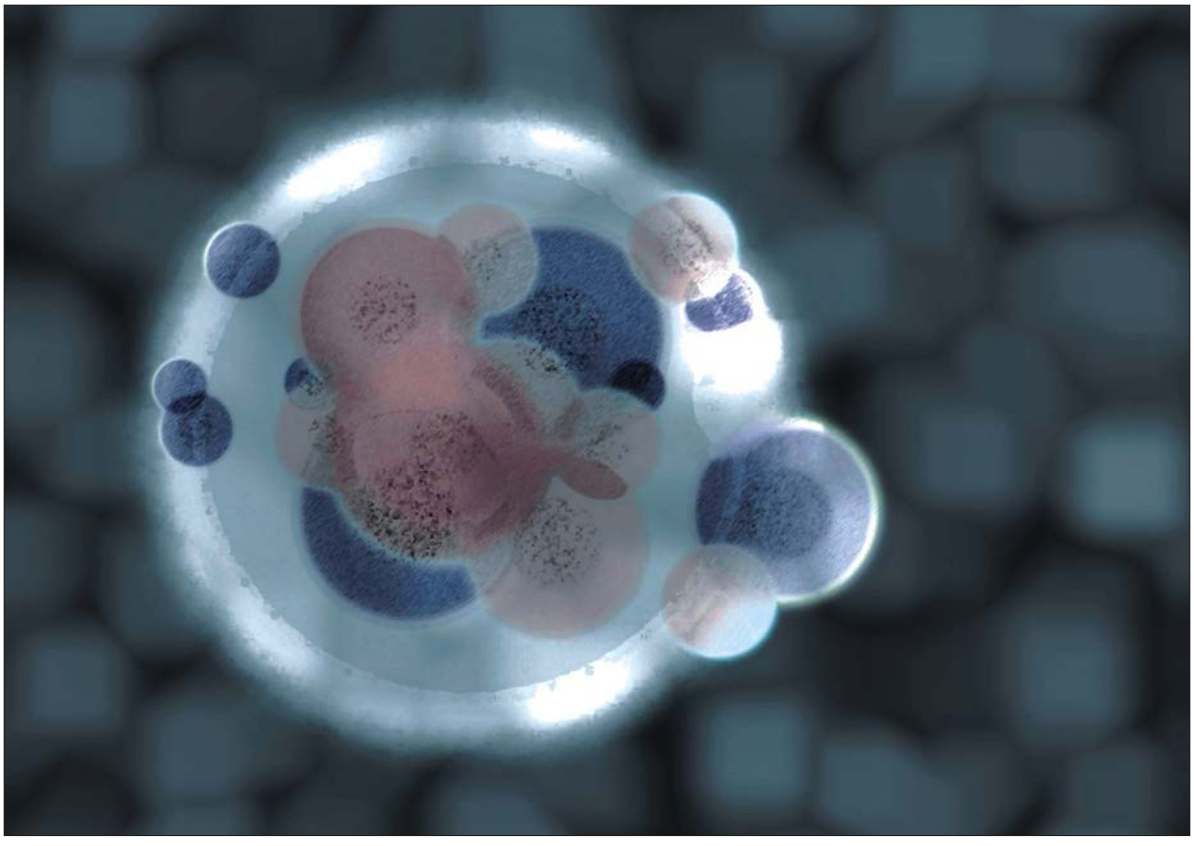

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Fungicide Seed Treatments Minimally Affect Arbuscular-Mycorrhizal Fungal (AMF) Colonization of Selected Vegetable Crops

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Abstract: Fungicides applied as soil drenches have been shown to adversely affect beneficial Arbuscular-Mycorrhizal Fungal (AMF) colonization of plant roots. We tested the effects of four common fungicides applied as seed treatments, mefenoxam, thiram, tebuconazole+metalaxyl and captan, on colonization of muskmelon (*Cucumis melo*), squash (*Cucurbita pepo* and *C. moschata*), bean (*Phaseolus vulgaris*), tomato (*Lycopersicon esculentum*) and corn (*Zea mays*) roots by the AMF *Glomus intraradices*. All inoculated seedlings were colonized with AMF, with treatment averages ranging from 6 to 99% root length containing hyphae and 0 to 68% containing vesicles. Overall, fungicidal seed treatment effects on AMF colonization were relatively minor and where significant effects were noted, they were inconsistent across species and/or sampling times. This study provides evidence that fungicidal seed treatments can be compatible with AMF inoculation and colonization.

Key words: Mycorrhiza, fungicide, seedling, seed treatment, colonization

INTRODUCTION

Growers who want to take advantage of beneficial natural or inoculated Arbuscular-Mycorrhizal Fungi (AMF) are understandably concerned about the possible negative effects of fungicides on AMF colonization of their seedlings. Soil drench applications of fungicides have been shown to impact AMF colonization of plant roots, with effects depending on the fungicide used, rate and, in some cases, also on the AMF species (Menge, 1982). Benomyl and Pentachloronitrobenzene (PCNB) tended to be more detrimental than captan in reducing AMF spore germination, hyphal growth and root colonization (Schreiner and Bethlenfalvay, 1997). Carbendazim, mancozeb, methoxy ethyl mercury chloride (Emisan), thiram and ziram all restricted mycorrhizal infection of groundnut (*Arachis hypogea*) (Sugavanam *et al*., 1994) and corn (*Z. mays*) (Menge, 1982). Fosetyl-Al reduced mycorrhizal colonization of onion *(Allium cepa)*, but phosphonate did not (Sukarno *et al*., 1998). Propiconazole applied at field rates had no detrimental effect on hyphal functioning (Schweiger and Jakobsen, 1998).

Formononetin, an isoflavone that has previously been shown to enhance AMF colonization, partially overcame the inhibition of AMF colonization by high soil P concentrations or herbicides (Fries *et al*., 1998; Siqueira *et al*., 1991a, b). It has not been reported whether formononetin might overcome inhibitory effects of fungicides on mycorrhizal fungal colonization.

We found no studies of how fungicides applied as seed treatments affect early AMF colonization of seedlings. Growers who want to inoculate seedling plants with AMF need to know whether seed treatments will hamper their efforts at obtaining mycorrhiza formation. Thus, in the present research the objective was to determine whether common fungicidal seed treatments impact AMF colonization and secondarily, whether formononetin could ameliorate possible negative impacts on colonization.

MATERIALS AND METHODS

Application of seed treatments: All treatments were prepared at label rates for seed treatment (Table 1), with distilled water used for preparation of slurries and drenches.

Muskmelon: Untreated Superstar F_1 muskmelon seed were obtained from Harris Seeds (Rochester, NY USA) and the following treatments applied: mefenoxam, mefenoxam+formononetin, tebuconazole+metalaxyl, tebuconazole+metalaxyl+formononetin, captan, captan+formononetin, formononetin, or control (no fungicide, no

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Table 1: Seed treatment products and application rates used in the study. All products were obtained from United States suppliers

Generic name	Fungicide name	Strength[a]	Company, City, State	Rate[b]	Application method
Mefenoxam	Apron XL	33.3%	Syngenta, Greensboro, NC	0.1 (g kg^{-1})	Slurry
Tebuconazole (T)	Raxil XT	15% (T)	Gustafson,	2.4 (mL kg^{-1})	
+metalaxyl (M)		20% (M)	Plano, TX		Slurry
Captan	Captan 50WP	48.9%	Bonide Products, Oriskany, NY	2.7 (mL kg^{-1})	Dust
Formononetin	Myconate	Not disclosed	VAMTech LLC, Lansing, MI	8 mg in 40 mL solution per container	Soil drench
Captan+chlorpyrifos (Lorsban) +streptomycin			Applied by Harris Seeds; formulation unknown		
Captan+thiram + metalaxyl			Applied by Harris Seeds; formulation unknown		
Captan			Applied by Harris Seeds; formulation unknown		
Thiram			Applied by Harris Seeds; formulation unknown		

[a] Percent active ingredient, [b] Active ingredient per unit seed

inoculum). Additionally, thiram-treated Superstar F_1 seed obtained from Harris Seed was tested with and without formononetin. Two seeds were planted in each of seven 40×180 mm containers per treatment. The containers were filled with a pasteurized sandy loam soil plus 50 mL whole soil inoculum containing *G. intraradices* spores and hyphae (mycorrhizal treatments), or 50 mL autoclaved inoculum (control).

Squash, tomatoes, corn, beans: In separate experiments, treated and untreated seed of the following cultivars were obtained from Harris Seeds: Provider green bean, Sweet Symphony sweet corn, Jet Star tomato, Zucchini Elite summer squash and Buttercup Burgess winter squash. Seed treatments were commercially applied seed treatments of captan+chlorpyrifos+streptomycin (bean); captan+thiram+metalaxyl (corn) and captan (tomato and squash); and laboratory applied seed treatments of mefenoxam, captan, tebuconazole+metalaxyl, or control (no fungicide). For each species x fungicide treatment, two seeds were planted in each of sixteen 40×180 mm containers. Half of these were filled with a pasteurized sandy loam soil plus 50 mL whole soil inoculum containing *G. intraradices* spores and hyphae (mycorrhizal treatment)and half with 50 mL autoclaved inoculum (mycorrhizal control). In a separate test, Jet Star tomato and Sweet Symphony corn seed were treated and planted as above, except that twelve containers were planted for each treatment and the control treatments consisted of (1) no fungicide x no mycorrhiza or (2) no fungicide x mycorrhiza. The purpose of this additional trial was to test for differences in colonization at an earlier stage of development.

Plant growth and harvest: The containers were arranged randomly within plant species and after emergence were thinned to one seedling per container. The plants were maintained in a greenhouse and after two weeks were fertilized once per week with a 50 mg kg^{-1} 14N-0P-14K-6Ca-3Mg (supplied by KNO_3, $CaNO_3$ and $MgNO_3$) solution.

All muskmelon plants were harvested at 30 days. Four plants of each treatment of winter squash, summer squash and tomatoes were harvested at 20 days and again at 32 days; beans and corn were harvested at 28 days and at 35 days. In the third trial, six plants of each treatment were harvested at 13 days (corn) or 22 days (tomatoes), with the remaining six harvested at 31 days (corn) or 36 days (tomatoes). Harvest dates of the third trial were determined by sampling additional inoculated control plants that had been included in the experiment specifically for the purpose of determining the early stages of colonization. Root and shoot fresh weights and shoot dry weights were recorded.

Colonization (hyphal and vesicle): Following harvest, roots were gently washed and frozen, then cleared in 10% KOH, acidified in 1% HCl, then stained with 0.05% aniline blue in 70% acidified glycerol (Grace and Stribley, 1991). Twelve 1.5 cm randomly selected root pieces from each plant were mounted onto slides and examined under 100x magnification for the presence of arbuscular-mycorrhizal hyphae and vesicles. Percent colonization was estimated by scoring ten sections per root piece for the presence or absence of hyphae or vesicles. Results within each experiment were subjected to analysis of variance (ANOVA) and means separated by Fisher's protected Least Significant Difference (LSD). Paired t-tests were used to compare the effect of the formononetin drench on colonization for each fungicide treatment of the muskmelons. Spearman rank and Pearson correlations were used to assess possible effects of the degree of colonization (percent hyphal or vesicle formation) on plant part weights.

RESULTS

The degree of colonization (percent root with hyphal or vesicle formation) was not significantly correlated with plant root or shoot weights in any of the trials (data not shown).

Muskmelon: By 30 days after planting, colonization was very high (>85% root length) in all inoculated treatments. None of the fungicide seed treatments significantly decreased hyphal length (data not shown) or vesicle formation (Fig. 1), compared with the non-fungicide (formononetin alone) control. In fact, the tebuconazole+ metalaxyl+formononetin treatment had significantly higher vesicle formation than the formononetin control. Formononetin drench of fungicide seed treatments increased vesicle numbers over the fungicides alone in all but the captan treatments, but not significantly. Without formononetin, vesicle formation was significantly higher in the captan and the tebuconazole+metalaxyl treatments than in the mefenoxam seed treatment (Fig. 1).

Other species: All inoculated plants were colonized by AMF. In the first trial, treatment averages of hyphal colonization ranged from 39% (tomato) to 99% (corn). Significant differences (Fig. 2) were found only at the second harvest dates of zucchini, corn and tomatoes. The differences were not consistent across the three species: in zucchini and corn, captan significantly lowered hyphal colonization compared to the untreated controls; in tomatoes the laboratory-applied captan as well as the tebuconazole+metalaxyl treatments had significantly higher colonization than either the control or the commercially-applied treatment. Treatment averages of vesicle formation ranged from 1% (tomatoes) to 68% (winter squash and beans). The only significant difference in vesicle formation at either harvest date was observed in the first zucchini sampling, where seedlings of mefenoxam-treated seeds had less than half the vesicle formation compared to the control (12% vs. 31%; p = 0.020).

In the second trial, treatment averages of hyphal colonization ranged from 6% (tomato) to 71% (corn). No significant treatment differences in hyphal colonization were observed in either species at any harvest of the second trial (data not shown). The 13-day old corn and both harvest dates of tomatoes exhibited minimal (<1%) vesicle formation across all treatments. In the 31-day corn, vesicle formation in the control (11%) and commercial (9%) seed treatments was significantly (p = 0.016) lower than that of the mefenoxam (26%) or tebuconazole+ metalaxyl (23%) treatments.

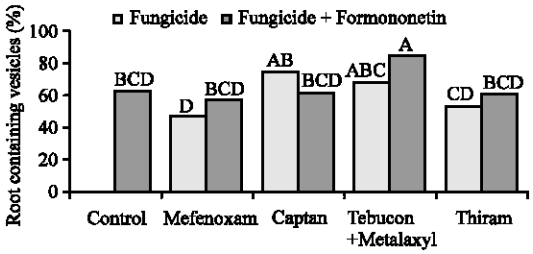

Fig. 1: Vesicle formation in muskmelon as influenced by fungicidal seed treatment. Different letters represent significant differences (p = 0.05) by Fisher's protected LSD.

DISCUSSION

Although mefenoxam, the metalaxyl formulation used here, is a systemic fungicide, it did not significantly decrease hyphal colonization by *G. intraradices*. Mefenoxam is a more purified version of the active isomer of the metalaxyl molecule that was previously shown to have mixed effects on AMF (Menge, 1982; Fontanet *et al*.,

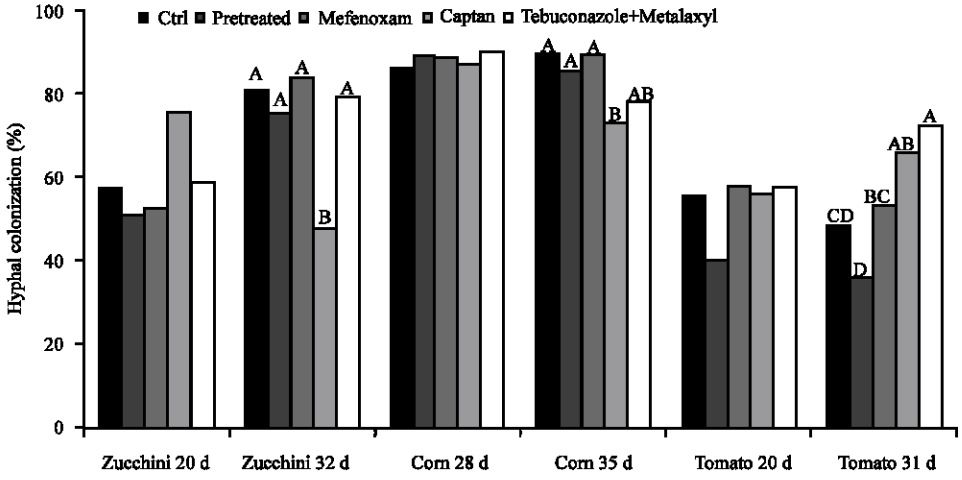

Fig. 2: Percent of seedling roots colonized by hyphae as influenced by fungicidal seed treatment and days after planting. The pre-treated fungicide treatments were: captan +thiram+metalaxyl (corn); captan (tomato and zucchini). Different letters within each species x day group represent significant differences (p = 0.05) by Fisher's protected LSD. Groups without letters did not differ significantly within the group

1998). Mefenoxam and metalaxyl are labeled to control watermolds, *Oomycetes* fungi such as *Phytophthora* and *Pythium* that can cause damping off of young seedlings. We found no reports of effects of soil or seed application of mefenoxam or of tebuconazole (xylem-mobile) on AMF. It appears that the mode of action of these systemic fungicides, sterol-biosynthesis inhibition active against membrane formation, is not one that is effective against the AMF *G. intraradices*, at least as a seed application. Only the protectant fungicide captan, designed to form a barrier between the germinating seed and attacking fungi, decreased hyphal colonization by *G. intraradices* and its effect was also inconsistent across studies and sampling times.

Vesicles are AMF structures filled with lipids and numerous nuclei, thought to function as energy storage organs for the fungi. The extent of vesicle formation by a given species of AMF is dependent on host, root and hyphal age and environment (Smith and Read, 1997) and generally increases under conditions favorable to hyphal colonization. Our data show that vesicle formation generally increased from the first to the second sampling, in accordance with van Aarle and Olsson (2003), who found that *G. intraradices* vesicle formation peaked at 30 days in *Plantago* roots. Again, fungicidal effects were relatively minor and inconsistent.

In summary, fungicidal seed treatment effects on AMF colonization were generally minor and where significant effects were noted, they were inconsistent across species and/or sampling times. Furthermore, except for the least developed corn and tomatoes, colonization treatment means (35% and above) were likely adequate to confer symbiotic effects on the host. Because some of these same fungicides have been shown to have deleterious effects on AMF when applied as a soil drench, it appears that the low rates needed for seed treatment dissipate sufficiently to allow for AMF to colonize the seedlings during early growth. Thus, this study provides evidence that fungicidal seed treatments can be compatible with successful AMF inoculation and colonization.

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