Effects of Inoculations with Mycorrhizal Fungi of Soilless Potting Mixes During Transplant Production on Watermelon Growth and Early Fruit Yield

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Abstract. Watermelon, *Citrullus lanatus* (Thunb.) Matsum. & Nakai, crops are continuously exposed to soilborne diseases. In many areas of the United States, greenhouse-raised watermelon seedlings are transplanted to the field to allow for early crop establishment and early fruit production. This practice can result in weakened root systems, which potentially make the plant prone to premature senescence and reduce crop productivity. Mycorrhizal fungi have been reported to improve plant growth in many crops through enhanced root growth and function. We hypothesized that amending potting mixes with commercial inocula of mycorrhizal fungi during seeding of watermelon in a greenhouse would improve watermelon production when seedlings were transplanted to the field. Colonization of watermelon roots with mycorrhizal fungi from three commercial formulations was compared with the colonization of onion roots to confirm the efficacy of the mycorrhizae. Two inocula of mycorrhizal fungi that resulted in colonization of watermelon roots were tested in the field and glasshouse for their potential to improve watermelon production. MycoApply improved early plant growth in two tests, one under *Meloidogyne incognita*-infested conditions in loamy sand and another at two phosphorus fertilizer levels (0 or 22 kg-ha⁻¹ P) in a loam soil. Mycor Vam Mini plug improved early fruit yield in soil infested with *M. incognita*. Application of Myconate (formononetin), a potential enhancer of colonization with mycorrhizae, increased early fruit yield in *M. incognita*-infested soil. Myconate had positive effects when potting mixes were not amended with inoculum of mycorrhizal fungi, but reduced watermelon growth when mycorrhizal fungi were supplied in the potting mix. In glasshouse tests, inoculation with mycorrhizal fungi did not suppress disease. Mycorrhizal fungi inoculations improved early plant establishment and increased the most valuable early fruit yield under some environmental stress conditions but did not increase total fruit yields.

In the United States, watermelon production occupied 59,000 ha in 2005, making it the fourth largest vegetable commodity (Anonymous, 2006). Intensive watermelon production is at risk for damage by soilborne

diseases such as Fusarium wilt caused by *Fusarium oxysporum* f. sp. *niveum*, mature watermelon vine decline (MWVD) caused by unknown agents, *Monosporascus* vine decline caused by *Monosporascus cannonballus*, root-knot nematodes, *Meloidogyne incognita*, and, in some areas, *M. javanica* (Brust et al., 1997; Bruton and Damicone, 1999; Egel and Latin, 2001; Egel et al., 2000, 2001, 2005; Martyn and Bruton, 1989; Martyn and Miller, 1996). Management strategies for these soilborne pathogens include crop rotation, covercropping, use of host plant resistance, soil fumigation, soil solarization, and nonfumigant chemical control (Egel, 2001; Hopkins and Elmstrom, 1984; Martyn and Hartz, 1986; Montalvo and Esnard, 1994; Thies and Levi, 2006a, 2006b; Westphal and

Egel, 2003; Zhou and Everts, 2006). Fields infested with *M. incognita* are of particular concern because of the wide host range of these plant parasites and the difficulty of managing them with cultural methods. Sustainable use of host plant resistance is limited by the lack of sources of resistance and by occurrence of races of pathogens with varying virulence patterns. More recently, amendments with rhizobacteria at seeding of watermelon into seedling trays, breeding for high root vigor, or grafting of susceptible watermelon scions on rootstocks of resistant cucurbits were examined to reduce infection with vine decline pathogens (Core, 2005; Crosby, 2000; Crosby et al., 2000; Kokalis-Burelle et al., 2003).

In many production areas, watermelon is produced with a transplant system on plastic mulch (Hochmuth et al., 2001). Seedlings are produced in peatmoss-based, soilless potting mixes in plastic trays. This production system allows for early and rapid establishment of 1-month-old transplants into plastic mulched seedbeds in the field at the beginning of the vegetation period (Hochmuth et al., 2001). Benefits of this practice include flexibility of the planting date during unpredictable weather conditions in spring and early fruit production (Olson et al., 1994; Vavrina et al., 1993). Root systems of transplanted watermelon plants may be weakened compared with those of seeded watermelon plants and can be predisposed to pathogen infection (D.S. Egel, pers. comm.; Elmstrom, 1973; NeSmith, 1999; Olson et al., 1994). Vigorous roots have mechanisms to cope with factors that cause stress (Miller, 1986), but transplanting may make cucurbit roots especially vulnerable when fruit set begins and fruits become the primary sink for photosynthates. In muskmelon, fruit removal resulted in extended vigorous vegetative growth of the plants compared with fruit-bearing plants (El-Keblawy and Lovett-Doust, 1996).

Mycorrhizal associations of vegetable roots can improve nutrient and water uptake and increase disease resistance compared with noncolonized roots (Linderman, 1988; Miller et al., 1986). Advances in aeroponics have overcome the obstacle that these obligate fungal endophytes cannot be grown axenically and commercial inocula are available (Hung and Sylvia, 1988; Jarstfer and Sylvia, 1992). These inocula are often used to amend potting mixes of high-value ornamental or crop plants (Anonymous, 2000). High levels of plant nutrients in agricultural systems have potential inhibitory effects on colonization with mycorrhizal fungi resulting from surplus nitrogen or phosphorus (Menge et al., 1978; Sylvia and Neal, 1990). The common practice of producing watermelon seedlings in peat-based potting mixes under controlled greenhouse conditions offers the opportunity to incorporate mycorrhizal fungus inoculations into the watermelon production system. Although it is known that *Glomus clarum* colonizes watermelon roots and improves fruit yield and water efficiency (Kaya et al., 2003), it is not known if watermelon

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roots are commonly colonized with mycorrhizal fungi. In greenhouse studies, positive growth protection from *M. incognita* under infested conditions was reported for muskmelon, *Cucumis melo* L. (Heald et al., 1989), but it is not clear whether colonization of watermelon roots with mycorrhizal fungi is beneficial under various environmental conditions and nutrient regimes in watermelon fields. Furthermore, there is a lack of information on whether colonization with mycorrhizal fungi can be enhanced with supporting methods. For example, formononetin, a plant biochemical involved in rhizobium nodulation of legumes, has been shown to promote rapid colonization with mycorrhizal fungi of some crops (Catford et al., 2006; Davies et al., 2005a, 2005b; Fries et al., 1996).

Objectives of this project were to: 1) determine whether amendment with commercial inoculants of mycorrhizal fungi to soilless potting mixes during watermelon seeding results in high colonization rates of watermelon; 2) determine if colonization of the seedlings in the greenhouse benefits watermelon under commercial production conditions; and 3) determine if applications of formononetin would have positive growth effects on watermelon under field conditions. The tests were conducted in fields with various nutrient or pathogen levels and in the glasshouse with artificial pathogen inoculations. Preliminary reports of these studies have been published (Snyder and Westphal, 2006; Westphal, 2007; Westphal et al., 2007; Xing et al., 2006).

Materials and Methods

Glasshouse tests for mycorrhizal colonization of watermelon roots. In a heated research glasshouse (25 to 27 °C, 14 h of light per day), three commercial mycorrhizal inocula and a nonamended control were tested in 36-cell commercial plastic trays (5 × 5.5 × 5.5 cm). A low-phosphate (9.2 mg·kg⁻¹ P) potting mix of 75% coarse peatmoss, 15% horticultural perlite, and 10% rockwool (BM 2; Berger Peat Moss, Saint-Modeste, Quebec, Canada) was used. Half of each tray was planted to watermelon 'Royal Sweet' and the other half was planted to onion 'White Lisbon'. Onion was included as a positive control for colonization potential of

the commercial formulations because of its known mycorrhizal status. Commercial inocula were powders containing survival structures of mixed species of mycorrhizal fungi at various spore concentrations (Table 1). Treatments included 1) nonamended control prepared by planting seeds into nonamended potting mix; 2) MycoApply (MAPP; Mycorrhizal Applications, Grants Pass, OR) with the powdery product applied on top of the seed after planting (4 g/cell in the first test and 1 g/cell in the second test); 3) Mycor Vam Mini plug inoculum (MVAM; Plant Health Care, Pittsburgh, PA), which was mixed with the potting mix before planting at 1.67 g·L⁻¹; and 4) 30 mL·L⁻¹ of Endomycorrhizal Inoculant-micronized (ENDO; BioOrganics, LaPine, OR), mixed with water, which was applied as a drench to each cell (30 mL per cell) and then seeded. Each cell received two seeds. Four replicate seedling trays were prepared for each treatment and the entire experiment was conducted twice (Test A and Test B).

Ten days after planting, seedlings were thinned to one per cell. Plants were fertilized weekly with modified Hoagland's solution (Hoagland and Arnon, 1950) devoid of P and with low concentrations of all other nutrients as needed. Five weeks after planting, five plants of each species were harvested at random from each tray, weighed, and the roots stained for determination of mycorrhizal colonization by the method of Brundrett et al. (1996). Root pieces from each seedling were washed gently and cut into 1-cm long pieces and mixed into a composite sample. Subsamples of 0.5 to 1 g of the root pieces were cleared for 8 min in boiling 10% KOH and transferred into 2% HCl for 20 min. The roots were washed with water and stained in boiling 0.05% trypan blue for 20 s and left to cool for 10 min. Roots were passed through destain solution (1 water : 1 glycerin : 1 lactic acid; by volume) twice and then stored in this solution until they were examined. The frequency of stained hyphae and other structures of fungi were counted microscopically and compared with root length based on the gridline intersect method (mean infection percentage method; Giovannetti and Mosse, 1980; Sylvia, 1994). Treatment effects on percent of stained roots were evaluated by analysis of variance (ANOVA), and least significant differ-

ences was used to differentiate means where appropriate.

Production of watermelon seedlings. Seedlings for two field trials and two glasshouse experiments were raised in plastic greenhouses, typical for commercial seedling production. One set of seedlings (Trial 1, Trial 2, and glasshouse Test I) was produced at the Southwest Purdue Agricultural Center (SWPAC) at Vincennes, IN (planted on 21 Apr.) and a second set at the Throckmorton Purdue Agricultural Center (TPAC) at Lafayette, IN (planted on 25 Apr.) for glasshouse Test II. Seedlings were raised in the BM2 potting mix as described previously. Similar procedures as described for the preliminary test were used to amend the inoculants of mycorrhizal fungi during seeding of watermelon with the exception that no onion checks were included, that plastic trays with 50 conical, round cells (5 cm top diameter, 2.5 cm bottom diameter, 6 cm deep) were used and that the ENDO inoculum was drench-applied after seeding (TPAC only). At transplanting to the field or to the glasshouse, mycorrhizal colonization of watermelon roots was determined as previously described.

Field trials. Two field trials were conducted near Vincennes, IN, in 2006. After field preparation with a combination of rototiller and field cultivator, black plastic film, standard material used to mulch watermelon beds, was placed on level (Trial 1: 18 m long plots = 17 plants) or 10 cm raised (Trial 2: 15 m long plots = 14 plants) seedbeds. Both trials were arranged in a randomized complete block design (Trial 1: five replications; Trial 2: four replications).

In Trial 1, watermelon growth was tested in *Meloidogyne incognita*-infested, loamy sand soil (84% sand, 12% silt, 4% clay, pH 5.7, 0.6% O.M.) typical for watermelon production fields of southern Indiana with 120 ± 12 mg·kg⁻¹ P, 110 ± 10 mg·kg⁻¹ K, and 430 ± 45 mg·kg⁻¹ Ca (as determined by standard procedures by A&L Great Lakes Laboratories, Fort Wayne, IN) and medium to high levels of second-stage juveniles (J2) of *M. incognita* (13 ± 1.3 J2/100 cm³ of soil at initiation of the trial on 27 Apr.; nematode identification confirmed by J. Eisenback, Virginia Polytechnic Institute and State University). Trial 1 received commercial applications of N, P, and K (Trial 1: 213 kg·ha⁻¹

Table 1. Commercial mycorrhizal inoculants, fungal species, and spore concentrations in each product according to label information.

Product	Company	Spore concn (spores/g or per cell) ^z	Mycorrhizal fungi
MycoApply, micronized endo (MAPP)	Mycorrhizal Applications	Total: 220/g ^z Single species: 73/g ^y Per cell: 220 ^y	<i>Glomus intraradices</i> , <i>G. mosseae</i> , <i>G. aggregatum</i>
Mycor Vam Mini plug (MVAM)	Plant Health Care	Total: 635/g Single species: 317/g Per cell: 73–160 ^x	<i>Entrophospora columbiana</i> , <i>Glomus intraradices</i>
Endomycorrhizal Inoculant (ENDO)	BioOrganics	Total: 67/g Single species: 8.3/g Per cell: 45	<i>Glomus aggregatum</i> , <i>G. clarum</i> , <i>G. deserticola</i> , <i>G. intraradices</i> , <i>G. monosporus</i> , <i>G. mosseae</i> , <i>Gigaspora margarita</i> , <i>Paraglomus brasilianum</i>

^zSpore concentrations were calculated based on the information on the product label.

^ySpore concentrations were calculated for a 1-g amendment amount.

^xSpore concentration dependent on cell size.

of N, 54 kg-ha⁻¹ of P, and 130 kg-ha⁻¹ of K). An irrigation line (t-tape; T-Systems International, San Diego, CA), 16 mm i.d., 20-cm emitter spacing, and an output of 500 LPH/100 m⁻¹ at 0.55 bar was installed. Two treatments were applied to suppress nematode population densities. One treatment was the application of a mix of 1,3-dichloropropene (1,3-D) and chloropicrin formulated for drip irrigation applications (InLine; Dow Agro-Sciences, Indianapolis, IN) at 168 L of formulation/ha on 27 Apr. 2006 through a contained system, injected with a piston pump (Inject-O-meter Mfg. Co., Clovis, NM) into the irrigation stream. Another treatment was oxamyl, formulated as Vydate L; it was applied through the drip irrigation system 10 d before planting (21 May 2006) at 2.26 kg-ha⁻¹ a.i. and 2 weeks after transplanting (13 June 2006) at 1.13 kg-ha⁻¹ a.i. in 250,000 L-ha⁻¹ of water at each application. At the postplant application, all other treatments received the same amount of water without the chemical.

In Trial 2, watermelon growth was tested at two different P fertilizer levels in a loam soil (44% sand, 39% silt, 17% clay, pH 6.8, 1.4% O.M.) with 47 ± 6 mg·kg⁻¹ P, 108 ± 8 mg·kg⁻¹ K, and 1325 ± 176 mg·kg⁻¹ Ca. In Trial 2, 55 kg-ha⁻¹ of K as KCl was applied to all plots, and 22 kg-ha⁻¹ of P was applied as triple superphosphate to the high P fertilizer plots. All plots received a uniform N application of 190 kg-ha⁻¹. The irrigation system had t-tape with 30-cm emitter spacing and an output of 165 LPH/100 m⁻¹.

During transplanting operations (Trial 1: 31 May; Trial 2: 1 June) in field plots, all treatments received water (Trial 1: 260 mL; Trial 2: 360 mL) to the planting hole ("setting water") that contained low concentrations of a starter fertilizer (N, P, K) and low concentrations of a soil insecticide (Trial 1). A minimum of five treatments of the transplants were established at each location: 1) control, nonamended potting mix; 2) MAPP added to the potting mix; 3) MVAM added to the potting mix; 4) MVAM added to potting mix and Myconate in the setting water; and 5) nonamended potting mix plus Myconate added to setting water. Myconate is a trade name product (Plant Health Care) of a potassium salt of formononetin. In Treatments 4 and 5, Myconate was applied with the setting water to deliver the equivalent of 1 mg to each plant hill. The 1-month-old watermelon seedlings, 'Royal Sweet', were planted at 105-cm spacing within the row. In Trial 1, there were the two additional treatments of 1,3-D or oxamyl applications for nematode suppression. Insect and foliar disease control applications followed local recommendations (Egel et al., 2006).

Four and 6 weeks after transplanting (28 June, 12 July), watermelon roots were removed from the soil with a shovel to a depth of 25 to 30 cm for visual inspections; care was taken to recover as much of the root system as possible. In Trial 1 at these dates, soil samples were also taken with an Oakfield soil sampling tube (19-mm diameter) to a 30-

cm depth into the root zone of the watermelon plants. Soil samples were used to extract J2 of *M. incognita* using Baermann funnels (Hooper, 1986). Root systems were examined for root-knot nematode infection at two sampling times: on 28 June, nematode-induced galls were counted; on 12 July and 16 Aug., severity of nematode-induced galling was rated (scale 0 to 10; Bridge and Page, 1980). Two weeks after planting (13 June), length of the main vine of the watermelon plants was measured. In Trial 2, watermelon plant vigor was rated on a scale of 1 to 5 (1 = very poor to 5 = very vigorous) on 28 June. At both locations, the youngest fully expanded leaves (12 per plot) were sampled during early flowering, and leaves were dried and processed for nutrient extraction by a commercial laboratory (A&L Great Lakes Laboratories Inc., Fort Wayne, IN). Top biomass was collected in both trials on 28 June. Fruit was harvested at two times (Trial 1: 31 July and 16 Aug.; Trial 2: 10 Aug. and 17 Aug.).

Watermelon growth in soil artificially infested with Meloidogyne incognita or mature watermelon vine decline in the glasshouse. For the glasshouse experiments, seedlings were produced in potting mixes amended with MAPP, MVAM, or ENDO inocula. Single 1-month-old seedlings were transplanted into 3-L plastic pots containing one of three different sandy soil mixes of autoclaved sand (95% sand, 3% silt, 2% clay, pH 7, 0.2% O.M.) amended with a loamy sand soil from a field with history of MWVD (79% sand, 15% silt, 6% clay, pH 6.5, 0.8% O.M.). Three different soil mixes of 90% autoclaved sand amended with 1) 10% autoclaved MWVD soil, 2) 10% nonautoclaved MWVD soil, or 3) 10% autoclaved MWVD soil infested with *M. incognita* (30,000 eggs per pot). The nematode eggs were collected from tomato greenhouse cultures of a single-egg-mass population originating from southern Indiana with standard procedures (Hussey and Barker, 1973). Each treatment was replicated six times in a randomized complete block design, and the entire experiment was conducted once with seedlings from SWPAC (Test I) and once with seedlings raised at TPAC (Test II) (the ENDO treatment was produced at TPAC only but used in both experiments). After 8.5 weeks, the tops were excised, oven-dried, and weighed. Roots were removed from soil, washed, weighed fresh, and rated for root-knot nematode damage. In addition to staining of plant roots at transplanting to the pots, root sections outside of the original seedling plug were excised and stained for the presence of mycorrhizae at harvest in the autoclaved mix (soil mix "a").

Data analysis. All data were analyzed with ANOVA. When transformations were necessary, these were applied. Nematode-related counts were log-transformed [$\log_{10}(x + 1)$]; rating data and colonization rates were arcsine-transformed [$\arcsine\sqrt{(x/\text{parameter maximum})}$] before being subjected to

ANOVA. Colonization rate means were compared with zero by *t* test.

Results

Colonization of watermelon roots with mycorrhizae. Roots were colonized with mycorrhizae in all trials. In the initial tests, colonization rates were higher in the MAPP treatment than in the MVAM treatment (Table 2). For the field trial seedling production, colonization rates were compared with the controls; some colonization was detected in all treatments, but only MAPP (with one exception) and ENDO treatments were consistently different from zero (Table 2).

Watermelon growth in Meloidogyne incognita-infested soil. In Trial 1, population densities of second-stage juveniles (J2) of *M. incognita* detected pre-season (data not shown), at planting, and 1 month after planting were low (Table 3). Most treatments had similar population densities of J2, except for the InLine treatment, which had some of the lowest numbers at planting, which were similar to Vydate L at planting. On 28 June 2006, these numbers were lower in the InLine treatment than in the control, but not different from those in MAPP or MVAM (Table 3). This same treatment had high population densities on 12 July 2006 (Table 3). At harvest, 16 Aug. 2006, all treatments had similar J2 population densities ranging from 300 to 695 J2/100 cm of soil and nematode-induced galling of watermelon roots ranged from 3.5 to 6.4 on a rating scale of 0 to 10 (data not shown).

Two weeks after transplanting, vine length was the longest for plants treated with Vydate L or MAPP, least for those treated with MVAM + Myconate, and intermediate in the other treatments (Table 3). At the early harvest on 31 July 2006, plants treated with MVAM and the control + Myconate had among the highest fruit yields and were similar to those treated with InLine. Yields of plants treated with InLine or Vydate L were not different from the control ($P = 0.0999$; Table 3). There were no significant treatment effects at the late harvest (data not shown) or on total yield (Table 3).

Watermelon growth at two different phosphorus fertilizer levels. In Trial 2, interactions of phosphate level × mycorrhizae treatment were not significant at $P = 0.05$, but the main effects of phosphate level or mycorrhizae affected several parameters. Watermelon vines, averaged across all treatments, were significantly longer in the high P treatment (21.2 cm) than those in the low P treatment (18.7 cm; $P < 0.01$), and watermelon growth was more vigorous at the higher P level (3.4 of a maximum score of 5) than at the low P level (2.5 of a maximum score of 5). Leaf tissue P concentrations on average were higher for plants in the low P soils (data not shown).

Plants treated with MAPP had longer vines than all other treatments when averaged across P treatments, and plants with MVAM + Myconate had the shortest vines (Table 4).

Table 2. Colonization of onion and watermelon roots with mycorrhizae from commercial formulations after 1 month of incubation in seeding trays in glasshouse experiments.^z

Treatment ^w	Initial tests for colonization potential (% of roots colonized) ^y				Watermelon seedlings for field and glasshouse experiments (% of roots colonized) ^x	
	Onion		Watermelon		SWPAC	TPAC
	Test A	Test B	Test A	Test B		
Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MAPP	28.2 ± 1.1 a*	4.9 ± 2.3*	33.7 ± 7.1 a*	52.9 ± 9.6 a*	15.3 ± 5.3	19.7 ± 5.6*
MVAM	5.7 ± 3.2 b*	2.2 ± 0.8	6.3 ± 3.2 b*	11.0 ± 0.6 b*	3.0 ± 0.5	2.3 ± 2.3
ENDO	17.4 ± 7.0 ab*	10.4 ± 5.8*	10.8 ± 3.6 b*	42.2 ± 3.2 a*	— ^v	23.3 ± 8.6*
<i>P</i> (arcsine-transformed)	0.0161	0.2829	0.0256	0.0053	0.1682	0.1652

^zNontransformed means (±SE) are presented. Means within each column were not significantly different by least significant difference when the arcsine-transformed data [$\arcsin\sqrt{(x/100)}$] were analyzed in analysis of variance at $P = 0.05$. Numbers indexed with an asterisk were significantly different from zero at $P = 0.05$ when tested by *t* test.

^yColonization in two glasshouse experiments each with four replications; controls were not included in the statistical analysis.

^xColonization of seedlings raised at Vincennes, IN (SWPAC) or Lafayette, IN (TPAC); two independent samples were processed from each location.

^wMAPP = MycoApply, micronized endo; MVAM = Mycor Vam Mini plug; ENDO = Endomycorrhizal Inoculant.

^vNot determined.

Table 3. Population densities of second-stage juveniles (J2/100 cm³ of soil) of *Meloidogyne incognita* and watermelon growth after various preplant soil drenches and amendments to planting plugs in an infested field at Vincennes, IN, in 2006 (Trial 1).^z

Treatment ^y	Soil population densities of <i>M. incognita</i> (J2/100 cm ³ of soil)			Plant growth and yield		
	At planting 31 May 2006	First sampling 28 June 2006	Second sampling 12 July 2006	Vine length (cm) ^x 13 June 2006	Early fruit wt/plant (kg) ^w 31 July 2006	Total fruit wt/plant (kg) ^w 31 July and 16 Aug. 2006
Control	16.4 ± 4.8 a	8.8 ± 1.2 a	116.4 ± 27.3	27.1 ± 1.3 b	7.1 ± 1.1 BC	9.6 ± 1.0
MAPP	10.0 ± 2.6 b	5.2 ± 1.0 ab	31.2 ± 10.8	31.5 ± 1.4 a	7.0 ± 1.3 BC	10.4 ± 1.1
MVAM	7.6 ± 1.8 bc	8.4 ± 2.1 a	85.0 ± 70.3	27.2 ± 1.3 b	9.0 ± 0.9 A	11.7 ± 1.4
MVAM + Myconate	10.4 ± 3.0 ab	8.8 ± 1.6 a	28.8 ± 7.0	22.8 ± 1.0 c	6.6 ± 0.8 BC	11.3 ± 0.9
Control + Myconate	9.6 ± 1.9 b	6.4 ± 3.0 ab	276.4 ± 123.3	26.9 ± 1.3 b	9.0 ± 1.0 A	13.6 ± 1.7
InLine	2.0 ± 1.3 c	2.0 ± 2.0 b	278.4 ± 152.7	29.8 ± 1.1 ab	8.0 ± 1.5 AB	11.5 ± 1.7
Vydate L	5.6 ± 1.7 bc	9.5 ± 4.9 a	52.8 ± 30.1	33.1 ± 1.4 a	6.0 ± 1.3 C	10.9 ± 1.9
<i>P</i>	0.0043 ^v	0.0054 ^u	0.1063 ^u	0.0004	0.0999	0.3666

^zMeans (± SE) within a column followed by the same letter were not significantly different at $P = 0.05$ (lower case letter) or at $P = 0.10$ (upper case letter).

^yMAPP = MycoApply, micronized endo; MVAM = Mycor Vam Mini plug; Myconate = setting water application of formononetin; InLine = mix of 1,3-D and chloropicrin at 168 L·ha⁻¹; Vydate L; oxamyl at 2.26 kg·ha⁻¹ a.i. before planting and at 1.13 kg·ha⁻¹ a.i. 2 weeks after planting.

^xLength of the main vine of the watermelon seedlings.

^wMarketable fruit weight: average per plant (kg) in plots of 11 to 13 plants.

^uProbability for analysis of variance and mean separation based on nontransformed means.

^vProbability for analysis of variance and mean separation based on log-transformed data [$\log_{10}(x + 1)$].

In the factorial analysis of the potting mix amendment with MVAM and planting hill amendment with Myconate, there was a highly significant interaction of the potting mix amendment and Myconate application ($P < 0.01$; means in Table 4). The main effects of the potting mix amendment ($P < 0.01$) and Myconate application ($P = 0.0150$) also significantly affected the vine length (means in Table 4). Watermelon growth in the MAPP treatment was more vigorous than in MVAM and MVAM + Myconate, but was similar to the control or control + Myconate ($P = 0.10$; Table 4). Top biomass was reduced in MVAM + Myconate compared with most other treatments (Table 4). In Trial 2, treatments did not affect fruit yields (Table 4).

Treatment affected leaf tissue P and K concentrations but not N levels (Table 4; N data not shown). The P concentrations in leaf tissues were lower after inoculation with MAPP than with MVAM averaged across P level of the soil, and both inoculations were not significantly different from the control (Table 4). Tissue K concentrations were higher in MVAM-inoculated plants (with or without Myconate) than in those inoculated with MAPP, which were similar to the control.

Watermelon growth in soil artificially infested with Meloidogyne incognita or

mature watermelon vine decline in the glasshouse. In glasshouse tests, mycorrhizal colonization did not affect severity of infection with *M. incognita* or MWVD; averaged for seedling plug treatments top dry weights were highest in the autoclaved soil (4.43 g), significantly lower in the MWVD soil (2.28 g) and in the *M. incognita*-infested soil (2.51 g). Effects of different mycorrhizal inoculations on plant growth were minimal and resulted in similar growth and vigor of non-colonized and colonized seedlings under glasshouse conditions (data not shown). In the autoclaved soil (soil mix “a”), root colonization rates at harvest with mycorrhizae outside of the original planting plug were high; colonization rates were the most variable for MVAM, a treatment that had either colonization rates as high as MAPP or lower rates more similar to ENDO (Table 5). In the soil series infested with *M. incognita*, final population densities of J2 ranged from 330 to 390 J2/100 cm³ and nematode-induced gall rating from 6.1 to 6.5, but there were no significant effects resulting from treatment.

Discussion

Colonization with mycorrhizal fungi of watermelon seedlings from commercial for-

mulations of inoculants of mycorrhizal fungi containing several species was documented, increasing the list of species shown to be capable of colonizing this crop. Colonization of watermelon roots with *Glomus* spp. in addition to the known colonizer *G. clarum* (Kaya et al., 2003) was confirmed. A more accurate determination of the specific species with colonization potential was not possible because of the mix of several species of mycorrhizal fungi in the products. When estimating the spore concentrations for the conical cells based on label information for each product, ≈220 spores per cell were added in the MAPP treatment (1 g product per cell), 106 spores per cell with MVAM, and 74 spores per cell with ENDO. It could be hypothesized that differences in colonized root length by the products were associated with spore concentrations of the fungi. However, differences in colonization rates were likely primarily the result of the composition of genera within the products and not the result of spore concentrations. Colonization with MAPP was consistently high. Under field conditions in sandy soils, plant growth- and yield-enhancing effects resulting from inoculations with mycorrhizal fungi were measured. Treatment with MAPP increased vine length in *M. incognita*-infested soil and

Table 4. Watermelon vine length, vigor rating, and plant top biomass after various amendments to the planting plugs and drench applications at planting in a field trial averaged across two phosphate fertility levels at SWPAC, Vincennes, IN, in 2006 (Trial 2).^z

Treatment ^x	Plant growth			Fruit wt/plant (kg) ^y		Leaf tissue nutrients	
	Vine length	Vigor rating	Top biomass (kg)	Early	Total 10 Aug. and	P (g·kg ⁻¹)	K (g·kg ⁻¹)
	13 June 2006 ^w	28 June 2006 ^v	28 June 2006 ^u	10 Aug. 2006	17 Aug. 2006		
Control	19.6 ± 0.7 b	3.0 ± 0.2 AB	1.5 ± 0.2 a	11.3 ± 0.5	11.5 ± 0.5	5.0 ± 0.1 ab	26.9 ± 0.4 bc
MAPP	26.4 ± 1.3 a	3.5 ± 0.3 A	1.7 ± 0.1 a	11.2 ± 0.6	11.5 ± 0.6	4.8 ± 0.1 b	26.6 ± 0.6 c
MVAM	18.3 ± 0.5 b	2.6 ± 0.2 B	1.2 ± 0.1 ab	11.0 ± 0.3	11.3 ± 0.3	5.2 ± 0.1 a	28.1 ± 0.4 a
MVAM + Myconate	14.4 ± 1.3 c	2.6 ± 0.4 B	1.1 ± 0.1 b	10.5 ± 0.8	11.4 ± 0.8	5.3 ± 0.1 a	28.8 ± 0.4 a
Control + Myconate	21.0 ± 0.9 b	3.0 ± 0.3 AB	1.5 ± 0.1 a	12.4 ± 0.4	12.7 ± 0.5	5.3 ± 0.1 a	27.8 ± 0.3 ab
P	< 0.01	0.0529	< 0.01	0.2763	0.5297	0.0107	0.0024

^zMeans (± SE) across the same mycorrhizae treatment indexed with the same letter were not significantly different at $P = 0.05$ (lowercase letter) or at $P = 0.10$ (upper case letter).

^yMarketable fruit weight: average per plant (kg) in plots of 11 of 13 plants.

^xMAPP = MycoApply, micronized endo; MVAM = Mycor Vam Mini plug; Myconate = drench treatment with a formulation of formononetin added to the "setting water" at planting.

^wLength of the main vine of the watermelon plant was measured and averaged per plot.

^vWatermelon plants were rated on a scale 1 to 5 (1: very poor to 5: very vigorously growing); original means presented, arcsine-transformed data [$\arcsine\sqrt{x/\text{parameter maximum}}$] used in the analysis.

^uAboveground plant biomass per seedling.

Table 5. Watermelon top dry weights in 1) autoclaved soil, 2) soil amended with MWVD soil, or 3) infested with *Meloidogyne incognita*; root colonization rates outside the original planting plug at harvest with mycorrhizae in autoclaved soil "a" in two glasshouse experiments (Test I: seedlings from SWPAC; Test II: seedlings from TPAC) in 2006.^z

Treatment ^y	Top dry wt (g) ^x	Colonization rates with mycorrhizae (%) ^w	
		Test I	Test II
Control	3.19 ± 0.22	0.0	0.0
MAPP	2.95 ± 0.18	63.2 ± 13.4 a*	48.7 ± 6.6 a*
MVAM	2.97 ± 0.19	53.1 ± 14.5 a*	15.9 ± 2.1 b*
ENDO	3.18 ± 0.22	1.7 ± 0.8 b	4.8 ± 2.1 b*
P	0.7038	0.0060	0.0058

^zNontransformed means (± SE) are presented. Means within each column were not significantly different by least significant difference when the arcsine-transformed data [$\arcsine\sqrt{x/100}$] were analyzed in analysis of variance at $P = 0.05$. Numbers indexed with an asterisk were significantly different from zero at $P = 0.05$ when tested by t test.

^yMAPP = MycoApply, micronized endo; MVAM = Mycor Vam Mini plug; ENDO - Endomycorrhizal Inoculant.

^xAverage of top dry weights of the two experiments and all soil treatments.

^wColonization rates determined as percent of total root length examined for the autoclaved soil (a) by the gridline intersect method. Analysis of variance was conducted on the arcsine-transformed data. Original data means are presented.

in noninfested soil at different P levels, but these differences did not result in increased fruit yields. In *M. incognita*-infested soil, amendment of potting mixes with MVAM or addition of Myconate in setting water increased early fruit yields compared with the nonamended control. Our field trials extended previous glasshouse studies with watermelon by other researchers that determined increased growth and some protection from the *Fusarium* wilt pathogen under greenhouse conditions (Ban et al., 2007; Li et al., 2000).

In controlled pot tests in the glasshouse, inoculants with mycorrhizal fungi did not reduce root-knot nematode infection or plant growth reductions resulting from MWVD. This confirmed previous observations on *Cucumis melo*, another root-knot nematode-sensitive cucurbit, that found limited effects of colonization with *Glomus intraradices* on nematode infection and reproduction, but a reduction of nematode damage by *M. incognita* was detected in mycorrhizal plants (Heald et al., 1989), an observation that was not confirmed in our studies with watermelon. Instead, numerical increases in nematode galling were detected, a result that

corresponds to earlier findings as summarized by Dehne (1981) that vesicular-arbuscular mycorrhizae increased nematode reproduction on soybean but contradicts findings with cucumber that showed reduced damage, infection, and reproduction of root-knot nematodes with mycorrhizae colonization (Dehne, 1981). Early yield enhancement by inoculations with mycorrhizal fungi under field conditions did not appear to be the result of suppression of root knot nematode infection in the field. No reduction of root knot nematode infection or detrimental effects of MWVD were observed in the glasshouse.

Effects on nutrient concentrations were found. Improvements of nutrient uptake, particularly of P, are highly desirable because of the soil chemical restrictions in P nutrition and the possible negative effects of current fertility programs in agricultural production (Miyasaka and Habte, 2001). Different mycorrhizal fungi explore the soil volume with their hyphae to different extents, and this can impact effectiveness of P assimilation because these structures facilitate nutrient transport partially by increasing the root surface area and root-soil contact (Hayman, 1981; Jakobsen et al., 1992). Other nutrient

uptake improvements are more general and are not mechanistically understood (Marschner and Dell, 1994; Ojala et al., 1983). Increases of P concentration in tissue as reported for some mycorrhizal symbioses (Ojala et al., 1983) were not found in this study. The high soil nutrient content in our trials could have reduced mycorrhizal colonization and effectiveness of the symbiosis as seen by Miyasaka and Habte (2001). On the other hand, mycorrhizal fungi can lead to the loss of function of direct P uptake by the root, resulting in complete dependence on the symbiont (Smith et al., 2003). Under high nutrient conditions, these interactions may mask benefits of the mycorrhizal association.

Neither specific disease-suppressing measures nor large plant nutrient benefits were found in our studies. The plant growth improvement must have been a more general plant growth-enhancing effect that was not quantifiable with the parameters measured. Perhaps general stress suppression was responsible for improvements in early plant growth and early fruit yield.

Colonization rates in a study with watermelon and *G. clarum* by Kaya et al. (2003) were higher than in our studies. However, the amount of inoculum in their study was much higher (30 g per plant hill) than in our study, and the application method of burying the inoculum underneath each plant hill was impractical for commercial use (Kaya et al., 2003). When we tested persistence of the fungi in watermelon roots grown in the autoclaved soil in the glasshouse, we found a high frequency of mycorrhizae outside of the original site of inoculation. It is not clear if the fungi grew along with the roots or if they recolonized through the soil. Nonetheless, this maintenance of colonization is beneficial, because it mitigates the problem of redistributing the beneficial microorganisms along the expanding root system throughout the season.

It appears that formononetin had some benefits with nonamended planting plugs, which confirmed the positive effects of this chemical on mycorrhizal colonization and nutrient uptake in potato (Davies et al.,

2005a, 2005b). Formononetin was detrimental for plants already colonized with MVAM before transplanting to the field. This material may offset the balance of the mycorrhization process as has been demonstrated in the role of formononetin in colonization of alfalfa roots with rhizobium and mycorrhizae (Catford et al., 2006). In those studies, formononetin was the mediator that affected inhibition of secondary colonization.

No attempt was made to determine which mycorrhizal fungi were the main colonizers. Such determination would be critical for mechanistic studies. This project attempted to evaluate commercial inocula of mycorrhizae for colonization of watermelon roots and the benefits of colonization for plant growth and productivity. The results indicate that mycorrhizal inoculations can enhance early growth and establishment of watermelon in instances when extreme environmental conditions aggravate transplanting stresses. The results do not suggest that making mycorrhizal inoculations a standard production practice will result in consistent productivity enhancements under all conditions.

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