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Short Communication

Persistence of *Metarhizium anisopliae* incorporated into soilless potting media for control of the black vine weevil, *Otiorhynchus sulcatus* in container-grown ornamentals

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Abstract

The objective of this study was to determine the persistence of *Metarhizium anisopliae* (F52), measured as infectivity against black vine weevil larvae, in a soilless potting medium at six wholesale nursery locations across the Willamette Valley, Oregon. A granule formulation (0.30 and 0.60 kg/m^3) was incorporated into media at planting and fungal persistence determined over two growing seasons. The fungus persisted in the potting media over the duration of the experiment with 50–60% of the larvae exposed to treated media becoming infected at the end of the experiment. The percentage of infected larvae gradually declined from $\geqslant$90% on week 3 to 40–60% by week 19. Larval infection rebounded over the fall and winter months of 2004 to 75–80% followed again by a slow decline over the course of the second growing season.
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Keywords: *Metarhizium anisopliae*; *Otiorhynchus sulcatus*; Microbial control; Fungal persistence

In the United States, the environmental horticulture industry (floriculture and nursery crops) is the third largest value crop behind corn and soybeans (USDA fact sheets, 2001; http://www.nass.usda.gov). The black vine weevil, *Otiorhynchus sulcatus* (F.) (Coleoptera: Curculionidae) is a univoltine, polyphagous insect that is a serious pest of field and container-grown landscape plants as well as small fruit crops worldwide (Moorhouse et al., 1992). The control program currently implemented by a majority of growers centers on the use of broad spectrum insecticides to target adults prior to oviposition. However, even when implementing an extensive spray program, growers often discover infested plant material. Infested plants cannot be sold and if infested plants are mistakenly shipped, the grower risks refusal by the buyer and will incur return shipping costs and potential loss of future sales. To help combat this pest, growers are escalating their use of soil incorporated insecticides at potting.

Metarhizium anisopliae (Metchnikoff) Sorokin (Hypocreales: Clavicipitaceae) has been studied extensively for the biological control of a wide range of insect pests, including black vine weevil (Moorhouse et al., 1992, 1993a,b; Booth and Shanks, 1998; Bruck, 2005). A new tool available to nursery growers for black vine weevil management is the incorporation of *M. anisopliae* (F52) into media at potting. This isolate is registered by the US Environmental Protection Agency, persists well in commercial peat and bark-based potting media (Bruck, 2005), as well as in commonly used potting media components (coir, peat, hemlock bark, fir bark and perlite) (Bruck, 2006). Different isolates of the same entomopathogenic fungus can have varying pathogenicity for a particular pest (Poprawski et al., 1985; Bruck, 2004) as well as respond differently to biotic and abiotic conditions. These factors make it important to focus research on an isolate that is commercially available.

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The objective of this study was to determine the persistence, measured as infectivity to black vine weevil larvae, of *M. anisopliae* (F52) in a large scale replicated field trial performed at wholesale nursery locations in Oregon, USA.

A bark-based potting medium, (OBC Northwest Nursery Mix #1, OBC Northwest, Canby OR, pH 5.3), consisting of 70% fine bark, 20% mulch, 10% pumice, typical of that used for container-grown plant production was used at all locations. A granular formulation of *M. anisopliae* (F52) (Novozymes Biologicals Inc., Salem, VA) was incorporated into media prior to potting. This product consists of a sporulated and dried culture of *M. anisopliae* on rice with a concentration of 1.7×10^9 spores/g formulated product.

Factorial experiments were arranged in a randomized complete block design with three replications. There were 14 containers (#1 [3.8 L], Anderson Die and Mfg. Co., Portland, OR 97222) in each replicate containing media incorporated with the formulated product at 0.30 and 0.60 kg/m^3 as well as an untreated control. The viability and concentration (spores/g) of the fungal product was assessed prior to incorporation (Goettel and Inglis, 1997). Treatments were produced using a concrete mixer, ran for 10 min, to uniformly incorporate the *M. anisopliae* into the potting media. In addition, 2.34 kg/m^3 of slow release fertilizer (Apex 14:14:14, J.R. Simplot Company, Lathrop, CA 95330) was incorporated into each treatment. Formulated treatments were transported from the laboratory to each nursery; six locations in total, and plants potted on site. Each grower provided plant varieties which are subject to black vine weevil infestations. Plant varieties were the same at each location but differed between locations. Plants were potted in April 2004 and placed in the production area at each nursery. The experiments were maintained for 17 months. During that time, plants were exposed to the same biotic (pest pressure, plant disease, etc.) and abiotic (light intensity, temperature, water availability, etc.) factors as the commercially grown plants at each location.

At all locations, a single container from each treatment was randomly selected per replicate during the following months in 2004 (May, June, July, August [2 samples] and September) and 2005 (January, March, May, June, July, August, September and October) and returned to the laboratory for processing. In the laboratory, plants were cut at the media surface and discarded. A golf hole corer (10.5 cm in diameter, Product # 41242, Reliable Golf Course Supplies, Queensbury, NY 12804) was used to remove the media from the center of each container, from the soil surface to the bottom of the container. The center core from each container and the outer ring (approximately 2.54 cm in width) of media were placed on separate metal trays and thoroughly mixed by hand. Infectivity was determined by taking a sample (~100 g) of the homogenized media from the center and outer ring of each container and placing it into a 150 ml plastic cup along with ten 6th instar black vine weevil obtained from a colony maintained at the USDA-ARS Horticultural Crops Research Laboratory (Fisher and Bruck, 2004). Cups were incubated at 24 °C for 14 days in complete darkness and assessed for larval infection.

The percentage of black vine weevil larvae infected with *M. anisopliae* (i.e., sporulating cadavers) on each sample date were analyzed using ANOVA. An arc-sine transformation of the square root of the percentage larval infection was performed to stabilize the variances (Snedecor and Cochran, 1989) and Tukey's multiple range test was used to separate means (SAS Institute, 1999). Abbott's formula was used to adjust for a small number of infected larvae (<25) found in the control treatments throughout the length of the experiment (Abbott, 1925). A test of homogeneity of variance was performed to detect variation between the trials performed at the various nursery locations (Little and Hills, 1978). Variability was not significantly different between the six trials and data were combined for analysis. Although samples were collected from each treatment over time, a repeated measures analysis was not required because containers were maintained individually, destructively sampled and fungal persistence from each container quantified only once. The significance level used for all statistical analysis was $P \leqslant 0.01$.

The effect of fungal treatment, container section sampled (center core or outer ring), sample week, and sample week by fungal treatment interaction significantly influenced the percentage of infected larvae (Table 1). There were no significant effects due to fungal treatment by container section interaction, sample week by container section sampled interaction or a sample week by fungal treatment by container section sampled interaction (Table 1). Because of the interaction between fungal treatment and sample week, the main effects of fungal treatment and sample week can not be compared directly (Cochran and Cox, 1992) and are presented only to provide a more complete overview of the data. The discussion of statistical differences between treatments is confined to the container section sampled and the fungal treatment by sample week interaction.

Metarhizium anisopliae (F52) persisted in the potting media over the duration of the experiment (two growing seasons). The lack of significant variation between locations, indicates that the isolate performed equally well regardless of location of each nursery or the known host plant species

Table 1
Results of an ANOVA analysis examining the effect of fungal treatment, pot section sampled, fungal treatment × pot section sampled interaction, sample week, sample week × fungal treatment interaction, sample week × pot section sampled interaction and sample week × fungal treatment × pot section sampled interaction on fungal infectivity towards black vine weevil

Source of variation	df	*F*	*P*
Fungal treatment	2	754.22	<0.0001
Pot section sampled	1	11.12	0.0009
Fungal treatment × pot section	2	4.16	0.02
Sample week	13	47.15	<0.0001
Sample week × fungal treatment	26	14.31	<0.0001
Sample week × pot section	13	0.69	0.77
Sample week × fungal treatment × pot section	26	0.47	0.99

utilized at each nursery. The mean (±SD) percentage of larval infection from all samples over the course of the experiment in the 0.30 and 0.60 kg/m^3 inoculation rates were 49.5 ± 32.6 and 56.7 ± 31.4, respectively. The overall mean percentage of larval infection differed significantly between media sampled from the inner core (56.16 ± 30.7) or outer ring (49.96 ± 33.4) of the containers. While statistically significant, it is unlikely that this difference represents any biologically significant difference in terms of larval infection and insect control in the field. The infectivity of media from the center core and outer ring from each inoculation rate followed the same pattern of decline over time (Fig. 1). The rate of decline was not statistically different between the center core and outer ring on any individual sample date ($F = 0.69$; df = 13, 1409; $P = 0.77$; Table 1), but over the course of the experiment the rate of decline was more pronounced in the outer ring. Media along the container perimeter would be exposed to temperature spikes; while media in the center would be insulated. Excessive temperatures in container-grown plants can result in root death, particularly in containers with southern exposure and no shade from surrounding containers (Whitcomb and Mahoney, 1984). Excessive temperatures also reduce fungal spore viability (Zimmermann, 1982). The use of pot-in-pot production systems moderate media temperatures and moisture fluctuations (Ruter, 1993) and its use may enhance fungal persistence. Studies are currently underway to determine the persistence of *M. anisopliae* (F52) in pot-in-pot production systems (Bruck, unpublished data).

The trend throughout the study at both inoculation rates was a decline in media infectivity from weeks 3 thru 77 ($y = -0.006x + 0.7623$; $R^2 = 0.60$). There was a significant sample week by fungal treatment interaction ($F = 14.31$; df = 26, 1409; $P < 0.0001$; Fig. 2). The percentage of larvae infected gradually declined from ⩾90% on week 3 (5/11/2004) to 40–60% by week 19 (8/31/2004). Infection levels rebounded over the fall and winter months (September–January) of 2004 to 75–80% followed again by a slow decline over the course of the second growing season to 30–40% (10/25/2005). The drop in fungal persistence observed in this study is somewhat typical of entomopathogenic fungi. A slow decline in fungal populations in both soil (Storey et al., 1989) and horticultural potting media is common (Bruck, 2005). Populations of *M. anisopliae* in soil under field conditions have been shown to decrease from 10^5 to 10^3 propagules/g soil after several months (Hu and St. Leger, 2002). The cause for the rebound in fungal infectivity over the winter months in our study is unclear. Fungal titers may have increased over the winter months due to the infection of naturally occurring weevil larvae sporulating in the containers. We did not observe any natural infections, but infected early instars decay relatively quickly after sporulation. These additional fungal propagules could have then been redistributed throughout the containers via rainfall. There were four sample dates (weeks 11, 19, 64, 68) with significant differences in the percentage of larval infection between the 0.30 and 0.60 kg/m^3 inoculation rates (Fig. 2). On each of those dates, significantly more larvae were infected at the high rate. On all other sample dates, there were no significant differences in the percentage of infected larvae between fungal treatments.

Other isolates of *M. anisopliae* incorporated into potting media also provided 90% control of black vine weevil

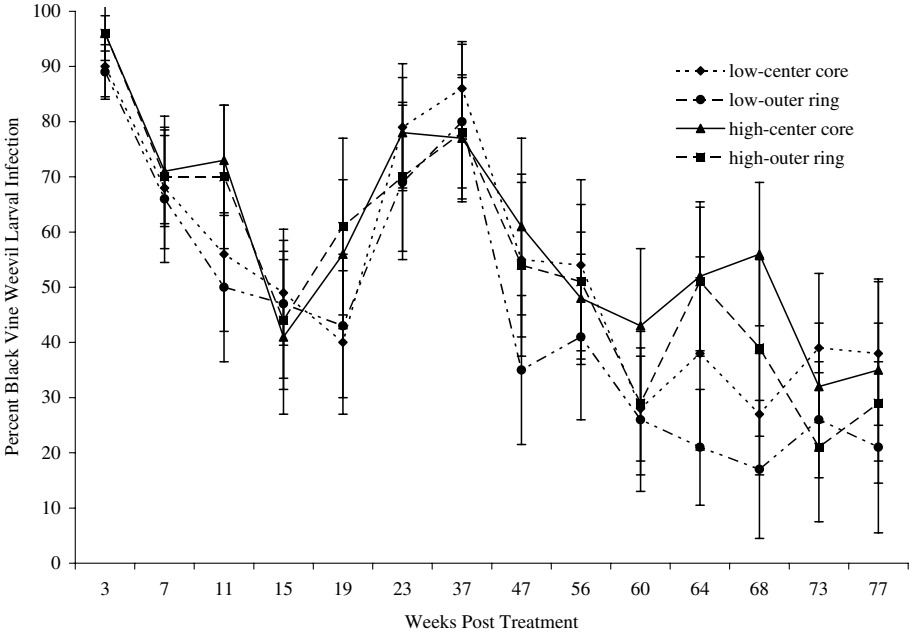

Fig. 1. Mean (±SD) percentage of black vine weevil larvae infected with *M. anisopliae* (F52) over all sample dates from the center core or outer ring in potting media incorporated with 0.30 (low rate) or 0.60 (high rate) kg/m^3 of formulated *M. anisopliae* (F52) granules.

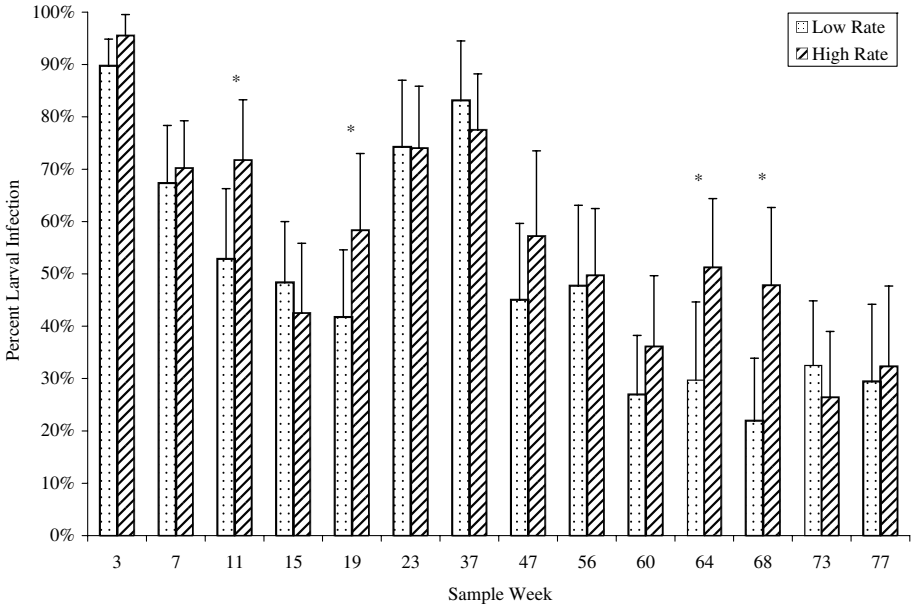

Fig. 2. Mean (±SD) percentage of black vine weevil larvae infected with *M. anisopliae* over all sample dates in potting media incorporated with 0.30 (low rate) or 0.60 (high rate) kg/m^3 of formulated *M. anisopliae* (F52) granules (corrected for control mortality; Abbott, 1925). Columns from the same date noted with an * indicate a significant difference in larval infection between inoculation rates ($P \leqslant 0.01$) (SAS Institute, 1999).

larvae in greenhouse grown ornamentals for up to 17 weeks (Moorhouse et al., 1993a). When used as a drench application, *M. anisopliae* applied at 1×10^9 conidia L^{-1} compost controlled 85–100% of black vine weevil larvae on a range of container-grown ornamentals (Moorhouse et al., 1993b). There is no significant difference in the efficacy of *M. anisopliae* (F52) incorporation (0.60 kg/m^3) to the industry standard bifenthrin incorporation (25 ppm) over the course of a single growing season (Bruck, unpublished data). Even though the level of infection observed at the end of the second growing season was relatively low (30–40%) the likelihood of a black vine weevil successfully completing its life cycle from egg to adult, a 9–10 month process, in a fungal inoculated container is small. Much is yet to be learned about the compatibility of this fungus with the chemical as well as other biological inputs used in containerized nursery production, but these and other data suggest (Bruck, 2005; Bruck, 2006) that *M. anisopliae* (F52) has the potential to control infestations of black vine weevil larvae for one or more growing seasons with a single incorporation at potting.

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