HRI's Mission:

To direct, fund, promote and communicate horticultural research, which increases the quality and value of ornamental plants, improves the productivity and profitability of the nursery and landscape industry, and protects and enhances the environment.

Effect of Potting Media Components on the Infectivity of *Metarhizium anisopliae* against the Black Vine Weevil (Coleoptera: Curculionidae)[1]

Denny J. Bruck[2]
USDA-ARS Horticultural Crops Research Laboratory
3420 N.W. Orchard Avenue, Corvallis, OR 97330

Abstract

The black vine weevil (BVW), *Otiorhynchus sulcatus* (F.) is a serious pest of nursery crops, particularly in the Pacific Northwest. The fungus, *Metarhizium anisopliae* (F52), has recently been registered by the U.S. Environmental Protection Agency for BVW control. The objective of this study was to determine the persistence of *M. anisopliae* in five common soilless potting media components (coir, fir bark, hemlock bark, peat moss and perlite), which was measured as pathogenicity against BVW larvae. Fungal granules (½ lb/yd^3) were incorporated with each media component at potting and fungal persistence determined for 133 days. Experiments were performed with and without plants to determine if the presence of a plant impacted fungal persistence. Overall, the fungus persisted well in all of the potting media components tested up to 133 days post application. Persistence was somewhat lower in the first experiment, likely due to fluctuating media moisture. In the second experiment, with more stable media moisture levels, the percentage of larval infection did not drop below 88% in any media at 133 days post application. It is likely that *M. anisopliae* will persist well and provide high levels of BVW larval control in most of the commercial potting media used in containerized nursery production, particularly those comprised primarily of the media components tested in these studies.

Index words: microbial control, biological control, pest management, soilless potting media, fungal persistence.

Species used in this study: *Picea abies* var. Nidiformis; Black vine weevil (*Otiorhynchus sulcatus* (F.)); *Metarhizium anisopliae* (F52).

Significance to the Nursery Industry

The black vine weevil (BVW), *Otiorhynchus sulcatus* (F.) is a serious pest of nursery crops, particularly in the Pacific Northwest. The BVW control program currently implemented by a large percentage of growers centers on the use of broad spectrum insecticides to target adults prior to oviposition. However, even when implementing an extensive insecticidal spray program, growers often discover plant material infested with last instars in the winter or in the spring prior to shipping. Infested plants cannot be sold and if infested plants are shipped, the grower risks refusal of the plants by the buyer and will incur the additional return shipping costs and potential loss of future sales. A new tool for BVW management is the incorporation of the entomopathogenic fungus *Metarhizium anisopliae* into the media at potting. This fungus has been recently registered by the U.S. Environmental Protection Agency and has been shown to persist and perform well in peat and bark-based potting media (2). What is not known, however, is how individual potting media components (coir, fir bark, hemlock bark, peat moss and perlite) impact fungal persistence. 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. However, these initial data suggest that *M. anisopliae* is compatible with common media components and has the potential to control BVW larval infestations for at least one or potentially more growing seasons with a single incorporation at planting.

[1]Recieved for publication September 23, 2005; in revised form January 5, 2006. I would like to thank Kelly Donahue for all of her hard work in sample processing and Amanda Lake for rearing the insects used in these experiments. This work was supported solely by the United States Department of Agriculture, Agricultural Research Service, Pacific West Area, Horticultural Crops Research Laboratory, Corvallis, Oregon, CRIS # 5358-22000-032-00D. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

[2]Research Entomologist.

Introduction

The black vine weevil (BVW), *Otiorhynchus sulcatus* (F.) (Coleoptera: Curculionidae) is a univoltine, polyphagous (over 140 host species) insect that is a serious pest of field and container grown landscape plants as well as small fruit crops worldwide (11). 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*). Oviposition occurs at night with eggs either dropped on the soil surface or inserted into crevices or on plants (21). Early instars begin by feeding on small roots while the later instars feed on larger roots, especially on the phloem and cambium tissues near the soil surface (9). Adults are nocturnal and cause mainly cosmetic damage to plants by notching the leaves. Adults reproduce by thelytokous parthenogenesis, so a single individual left unchecked can result in the infestation of an entire nursery.

Recently, the entomopathogenic fungus *Metarhizium anisopliae* (F52) (Metchnikoff) Sorokin (Hypocreales: Clavicipitaceae) received registration from the U.S. Environmental Protection Agency for use against BVW. *Metarhizium anisopliae* has been studied extensively for the biological control of a wide range of insect pests, including BVW (1, 2, 3, 12, 13, 15) and various other soil-borne pests such as *Popillia japonica* (Coleoptera: Scarabaeidae) (24), *Ligyrus subtropicus* (Coleoptera: Scarabaeidae) (16), *Antitrogus parvulus* (Coleoptera: Scarabaeidae) (18) and *Adoryphorus couloni* (Coleoptera: Scarabaeidae) (17). However, until now there was not an isolate of *M. anisopliae* that could be tested for BVW control that was registered for com-

mercial use. Different isolates of the same entomopathogenic fungus can have varying pathogenicity to a particular pest (1) as well as respond differently to biotic and abiotic conditions. These factors make it important to focus research on an isolate that may soon be commercially available. The use of *M. anisopliae* and other entomopathogenic fungi for control of soil-borne insects is often inconsistent due to limited spore redistribution and persistence (23). Biological control agents differ fundamentally from chemical agents in that in order to be effective, they must proliferate in the environment they are introduced (14). An understanding of the impact of soilless potting media components on the ability of *M. anisopliae* to maintain an efficacious population and persist in the environment where control is desired would improve biological control efficacy.

The objective of this study was to determine the persistence, measured as efficacy against BVW larvae, of *M. anisopliae* in five (coir, fir bark, hemlock bark, peat moss and perlite) common components of soilless potting media.

Materials and Methods

Five types of soilless potting media components typically used in container-grown nursery production were used. The components studied were coir (Coco Palm Resources, Hillsboro, OR), fir bark (Rod McLellan Co., Independence, OR), hemlock bark (Rexius Forest Products, Eugene, OR), Canadian sphagnum peat moss (Sun Gro Horticulture, Bellevue, WA) and perlite (Supreme Perlite Co., Portland, OR). The granule formulation of *M. anisopliae* (strain F52) (Earth BioSciences, New Haven, CT) was used. The formulated fungal product consisted of *M. anisopliae* that had conidiated on rice grains and was then dried. The concentration of conidia on the formulated product was 2.66×10^9 conidia/g.

The factorial experiments were arranged in a completely randomized design with four replications. There were two pots (8.9 × 8.9 × 8.9 cm (3.5 × 3.5 × 3.5 in), T.O. Plastics Inc., Minneapolis, MN, Item # 07090) in each replicate containing each media incorporated with *M. anisopliae* spores at the recommended rate of ½ lb/yd^3 and one untreated control pot of each media component with and without a rooted cutting of *Picea abies* (L.) Karst. (Pinales: Pinaceae) 'Nidiformis' for each sample date. The fungal isolate used in these studies colonizes the rhizosphere of *P. abies* 'Nidiformis' as evidenced by fungal population in the rhizosphere soil being significantly greater in the surrounding bulk soil (2); therefore, the experiment was performed in each media with and without plants to determine if fungal persistence was enhanced when roots were present in the media. The viability of the fungal product was assessed prior to incorporation (7) and the granule incorporation rate adjusted as necessary. Spore viability was 90–95%. All experimental treatments were incorporated by first mixing the recommended rate of *M. anisopliae* required for one replicate into 3 liters of media in a twin shell blender (Patterson-Kelley, East Stroudsburg, PA) for 7 minutes. This volume of media was then mixed with remaining media in a concrete mixer for 10 minutes to ensure that the fungus and the media were incorporated uniformly. Pots were filled with media from each treatment and maintained in a screenhouse where they were exposed to ambient conditions. Monthly, pots from each treatment were randomly selected (two fungal inoculated pots both with and without a plant as well as a single control pot with and without a plant from each replicate) and the infectivity of the fungus determined. Fungal infectivity was determined by taking a sample of soil from the center of each pot and placing it in a 5 oz plastic cup along with ten 6th instar BVW obtained from a colony maintained at the USDA-ARS Horticultural Crops Research Laboratory (6). Cups were incubated at 24C (75F) for 14 days at which time the numbers of live and *M. anisopliae* infected larvae were determined. Samples were collected 8, 28, 56, 77, 105 and 133 days post fungal application from the first experiment and days 28, 56, 77, 105 and 133 from the second experiment. The first experiment was performed from May–September 2004 and the second experiment from October 2004–February 2005.

The percentage of BVW larvae infected with *M. anisopliae* (i.e. sporulating cadavers) from the treatments on each sample date were analyzed using the General Linear Models Procedure. An arc-sine transformation of the square root of the percentage larval infection was performed to stabilize the variances (22) and Tukey's multiple range test was used to separate means (19). The variability of the data between experiments precluded their being combined for a single analysis and data from each experiment were analyzed separately (4). Although samples were collected from each treatment over time, a repeated measures analysis was not required because pots were maintained individually, destructively sampled and fungal persistence from each pot quantified only once.

Results and Discussion

The statistical analysis of the data from each run of the experiment resulted in the same sources of variation being significant ($P < 0.05$). The effect of media component, sample day and the media component by sample day interaction significantly influenced the percentage of fungal infected larvae in each experiment. There were no significant effects due to the presence of a plant in the pot, or media component by plant presence interaction on the percentage of infected larvae in either experiment. Because of the interaction between media component and sample day, the main effects can not be compared directly (4) and are presented only to provide a more complete overview of the data. The discussion of statistical differences between treatments will be confined to the media component by sample day interaction for each experiment.

Overall, *M. anisopliae* persisted well in all of the media components tested. The percentage of larval infection observed from the potting media components tested never dropped below 75% and was rarely less than 85% thru both experiments (Table 1). This fungal isolate has also been shown to persist well in both commercial peat and bark-based potting media for nearly one year (2). The overall percentage of larval infection throughout the length of the study was high, particularly in the second experiment (Table 2). Infection levels were lower on samples collected on days 77, 105 and 133 in the first experiment, which coincided with July, August and September of 2004. Average daily high air temperatures at this time averaged 29.0, 28.5 and 21.67C (84.25, 83.31 and 71F), respectively. Even with daily watering, it was difficult to maintain soil moisture in the small pots used in the study, chiefly in July and August. This was particularly true for the perlite treatment, which was often watered twice daily. The fluctuations in soil moisture are also the likely cause for the near lack of infection in the perlite treatment on day 77

Table 1. Mean (±SD) percentage of black vine weevil larvae infected with *M. anisopliae* overall dates among potting media components incorporated with ½ lb/yd³ of formulated *M. anisopliae* granules.

	Mean percentage of infected black vine weevil larvae	
Media component	Experiment 1	Experiment 2
Coir	86.95 (19.13)	94.38 (8.69)
Fir bark	75.96 (29.81)	90.75 (11.88)
Hemlock bark	82.50 (23.40)	94.43 (9.02)
Peat	86.35 (21.33)	92.50 (9.61)
Perlite	80.83 (36.61)	91.02 (13.92)

Table 2. Mean (±SD) percentage of black vine weevil larvae infected with *M. anisopliae* among all potting media types incorporated with ½ lb/yd³ of formulated *M. anisopliae* granules at each sample date.

	Mean percentage of infected black vine weevil larvae	
Day	Experiment 1	Experiment 2
8	99.13 (3.25)	—[z]
28	96.88 (6.67)	93.13 (9.08)
56	97.69 (5.79)	88.88 (13.87)
77	51.89 (31.17)	96.33 (6.64)
105	78.38 (25.50)	90.63 (12.94)
133	71.25 (29.99)	94.13 (8.67)

[z]Sample not taken.

(July) of the first experiment (Table 3). In fact, infection levels in all treatments dipped on the sample collected on day 77 of the first experiment. However, the infection levels nearly rebounded to their previous levels. Even in the perlite treatment, where the fungus was barely detected at day 77, infection levels rebounded to >90% on days 105 and 133. Fir bark was the only potting media that adversely impacted fungal infectivity. The reduction in infectivity was observed in both experiments, but not until samples collected 105 and 133 days after fungal incorporation. The reduction was most pronounced in the first experiment and may have been compounded by the fluctuating moisture levels as outlined previously. However, in complete potting media comprised primarily of fir bark, *M. anisopliae* persisted well for nearly one year (2).

Even though this fungal isolate has been shown to colonize the rhizosphere of *P. abies* (2), the presence of *P. abies* in pots containing the various media components did not significantly influence fungal persistence. The rhizosphere is the zone of soil immediately adjacent to plant roots. Rhizosphere competent microorganisms are defined as those that show enhanced growth in response to developing roots or a classical rhizosphere effect (20). The population increase due to rhizosphere colonization by *M. anisopliae* did not translate into differences in fungal infectivity in the bulk media. Entomopathogenic fungi do not persist well in field soils (8, 10, 23). Populations of *M. anisopliae* in bulk soil under field conditions have been shown to decrease from 10^5 to 10^3 propagules/g soil after several months (8). Factors such as soil temperature (5, 10) and high levels of soil moisture can reduce fungal persistence. Decline of *M. anisopliae* in wet soils (0 and –2.0 kPa) occurs at 30 and 60 days in clay and sandy loam soil, respectively (10). The use of *M. anisopliae* and other entomopathogenic fungi for control of soil borne insects is also often limited and inconsistent due to limited spore redistribution and persistence in field soils (23).

It appears that all of the potting media components tested in these experiments were compatible with the use of *M. anisopliae* for control of BVW larvae. The level of larval infection observed in each of the media components up to 133 days post application were high, particularly in the second experiment. The lower levels of infection observed in the first experiment from days 77, 105 and 133 were likely due to fluctuating moisture levels. Fungal persistence remained high on all sample dates performed in the second experiment when soil moisture was easier to maintain. In a wholesale nursery setting, soil moisture is well maintained throughout the growing season and would likely not be a cause for declining fungal persistence. Field studies have also shown that this fungal isolate is also infective for up to 24 months in container-grown plants maintained outdoors (Bruck, unpublished data), as well as the number of colony

Table 3. Mean (±SD) percentage of black vine weevil larvae infected with *M. anisopliae* at each sample date in each potting media component incorporated with ½ lb/yd³ of formulated *M. anisopliae* granules.

		Mean percentage of infected black vine weevil larvae	
Day	Media component	Experiment 1	Experiment 2
8			
	Coir	98.13 (4.03)a[z]	—[y]
	Fir bark	98.75 (3.42)a	—
	Hemlock bark	100.00 (0.00)a	—
	Peat	98.75 (5.01)a	—
	Perlite	100.00 (0.00)a	—
28			
	Coir	96.25 (5.00)a	95.00 (6.33)a
	Fir bark	96.25 (8.90)a	92.50 (10.64)a
	Hemlock bark	98.13 (5.41)a	93.75 (7.18)a
	Peat	98.13 (5.41)a	92.50 (11.25)a
	Perlite	95.63 (8.12)a	91.88 (9.81)a
56			
	Coir	99.38 (2.50)a	95.00 (8.16)a
	Fir bark	96.43 (8.43)a	92.50 (7.74)a
	Hemlock bark	97.50 (5.80)a	96.88 (7.93)a
	Peat	97.50 (5.80)a	88.75 (10.88)a
	Perlite	97.50 (5.80)a	71.25 (15.86)b
77			
	Coir	58.00 (23.41)a	95.63 (7.27)a
	Fir bark	65.63 (18.91)a	95.63 (7.27)a
	Hemlock bark	66.25 (15.86)a	96.88 (7.04)a
	Peat	67.50 (23.52)a	96.88 (6.02)a
	Perlite	2.50 (7.70)b	96.67 (6.17)a
105			
	Coir	85.63 (15.51)a	89.38 (13.40)ab
	Fir bark	59.67 (33.40)b	84.38 (15.98)b
	Hemlock bark	73.75 (25.00)ab	88.67 (13.56)ab
	Peat	81.25 (19.28)a	91.25 (10.88)ab
	Perlite	91.88 (20.07)a	99.38 (4.43)a
133			
	Coir	82.50 (15.71)a	96.88 (4.79)a
	Fir bark	41.88 (29.26)c	88.75 (13.60)b
	Hemlock bark	59.38 (27.68)bc	95.63 (6.29)ab
	Peat	75.00 (29.66)b	93.13 (6.29)ab
	Perlite	97.50 (5.78)a	96.25 (7.19)a

[z]Means followed by the same letter on the same sample date within a column are not significantly different ($P < 0.05$) (19).

[y]Sample not taken.

forming units remaining relatively stable in peat and bark-based media for nearly one year (2). It is likely that this fungal isolate will persist well in most of the potting media used in containerized plant production, particularly those comprised primarily of the media components tested in these studies. 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 initial data suggest that *M. anisopliae* has the potential to control infestations of BVW larvae for one or more growing seasons with a single incorporation at planting.

Literature Cited

1. Bruck, D.J. 2004. Natural occurrence of entomopathogens in pacific northwest nursery soils and their virulence to the black vine weevil, *Otiorhynchus sulcatus* (F.) (Coleoptera: Curculionidae). Environ. Entomol. 33:1335–1343.

2. Bruck, D.J. 2005. Ecology of *Metarhizium anisopliae* in soilless potting media and the rhizosphere: implications for pest management. Biol. Control 32:155–163.

3. Booth, S.R. and C.H. Shanks Jr. 1998. Potential of a dried rice/mycelium formulation of entomopathogenic fungi to suppress subterranean pests in small fruits. Biocontrol Sci. Technol. 8:197–206.

4. Cochran, W.G. and G.M. Cox. 1992. Experimental Designs. John Wiley & Sons, New York, NY.

5. Ekesi, S., N.K. Maniania, and S.A. Lux. 2003. Effect of soil temperature and moisture on survival and infectivity of *Metarhizium anisopliae* to four tephritid fruit fly puparia. J. Invert. Pathol. 83:157–167.

6. Fisher, J.R. and D.J. Bruck. 2004. A technique for continuous mass rearing of the black vine weevil, *Otiorhynchus sulcatus*. Entomol. Exp. Appl. 113:71–75.

7. Goettel, M.S. and G.D. Inglis. 1997. Fungi: Hyphomycetes. p. 211–249. *In*: L.A. Lacey (Editor). Manual of Techniques in Insect Pathology. Academic Press, San Diego.

8. Hu, G. and R. St. Leger. 2002. Field studies using a recombinant mycoinsecticide (*Metarhizium anisopliae*) reveal that it is rhizosphere competent. Appl. Environ. Microbiol. 68:6383–6387.

9. La Lone, R.S. and R.G. Clarke. 1981. Larval development of *Otiorhynchus sulcatus* (Coleoptera: Curculionidae) and effects of larval density on larval mortality and injury to rhododendron. Environ. Entomol. 10:190–191.

10. Li, D.P. and D.G. Holdom. 1993. Effect of soil matric potential on sporulation and conidial survival of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes). J. Invert. Pathol. 62:273–277.

11. Moorhouse, E.R., A.K. Charnely, and A.T. Gillespie. 1992. Review of the biology and control of the vine weevil, *Otiorhynchus sulcatus* (Coleoptera: Curculionidae). Ann. Appl. Biol. 121:431–454.

12. Moorhouse, E.R., M.A. Easterbrook, A.T. Gillespie, and A.K. Charnley. 1993. Control of *Otiorhynchus sulcatus* (Fabricius) (Coleoptera: Curculionidae) larvae on a range of hardy ornamental nursery stock species using the entomogenous fungus *Metarhizium anisopliae*. Biocontrol Sci. Technol. 1:63–72.

13. Moorhouse, E.R., A.T. Gillespie, and A.K. Charnley. 1993. Application of *Metarhizium anisopliae* (Metsch.) Sor. conidia to control *Otiorhynchus sulcatus* (F.) (Coleoptera: Curculionidae) larvae on glasshouse pot plants. Ann. Appl. Biol. 122:623–636.

14. Nelson, E.B., L.L. Burpee, and M.B. Lawton. 1994. Biological control of turfgrass diseases. p. 409–427 *In*: A. Leslie (Editor). Handbook of Integrated Pest Management for Turf and Ornamentals. CRC Press, Boca Raton, FL.

15. Poprawski, T.J., M. Marchal, and P.-H. Robert. 1985. Comparative susceptibility of *Otiorhynchus sulcatus* and *Sitona lineatus* (Coleoptera: Curculionidae) early stages to five entomopathogenic Hyphomycetes. Environ. Entomol. 14:247–253.

16. Raid, R.N. and R.H. Cherry. 1992. Effect of soil parameters on pathogenicity of the fungus *Metarhizium anisopliae* to the sugarcane grub *Ligrus subtropicus* (Coleoptera: Scarabaeidae). Fla. Entomol. 23:179–184.

17. Rath, A.C. 1988. Developments in the use of *Metarhizium anisopliae* for control of subterranean red-headed cockchafer (Coleoptera: Scarabaeidae: *Adoryphorus couloni*). p. 88–95 *In*: P.P. Stahle (Editor). Proc. 5th Austral. Conf. Invert. Ecol., Melbourne Univ. D & D Printing, Victoria.

18. Samuels, K.D.Z., D.E. Pinnock, and R.M. Bull. 1990. Scarabaeid larvae control in sugarcane using *Metarhizium anisopliae*. J. Invertebr. Pathol. 55:135–137.

19. SAS Institute. 1999. The SAS Statistical System, version 8. SAS Institute, Cary, NC.

20. Schmidt, E.L. 1979. Initiation of plant root-microbe interactions. Ann. Rev. Microbiol. 33:355–376.

21. Smith F.F. 1932. Biology and control of the black vine weevil. USDA Technical Bulletin 325.

22. Snedecor, G.W. and W.G. Cochran. 1989. Statistical Methods, eighth ed. Iowa State University Press, Ames.

23. Storey, G.K, W.A. Gardner, and E.W. Tollner. 1989. Penetration and persistence of commercially formulated *Beauveria bassiana* conidia in soil of two different tillage systems. Environ. Entomol. 18:835–839.

24. Villani, M.G., S.R. Krueger, P.C. Schroeder, F. Consolie, N.H. Consolie, L.M. Preston-Wilsey, and D.W. Roberts. 1994. Soil application effects of *Metarhizium anisopliae* on Japanese beetle (Coleoptera: Scarabaeidae) behavior and survival in turfgrass microcosms. Environ. Entomol. 23:502–513.