

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO	
RAD: 14-274204- -1	FECHA: 2015-05-18 17:01:54
DEP: 2020 DIRECCION DE NUEVAS CREACIONES	EVE: 378 FASE NACIONAL INCLUIDO CAPITULO I
TRA: 11 PCT-PATENTE DE INVENCION CAPITULOS I Y II	FOLIOS: 24
ACT: 519 TRASLADOINFORME	

DIRECCIÓN DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogado(a)/Señor(a)
JAIME EDUARDO DELGADO VILLEGAS

Asunto: Radicación: 14-274204- -1
 Trámite: 11
 Evento: 378
 Actuación: 519
 Oficio: 5432
 Folios: 24

Patente de Invención	X			Patente de Modelo de Utilidad	
Vía Nacional					
Vía PCT (Fase Nacional)	X	Capítulo I	X	Capítulo II	
No. Solicitud Internacional	PCT/EP2011/071418				
Fecha de Presentación Internacional	30/11/2011				

Como resultado del examen de fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripción:	Radicado 14-274204-00000-0000	12/Diciembre/2014
Reivindicaciones:	Radicado 14-274204-00000-0000	12/Diciembre/2014
Prioridad:	EP 10193335.6	01/Diciembre/2010
	US 61/419,438	03/Diciembre/2010

2. Análisis de la Prioridad

Se acepta la reivindicación de prioridad porque ésta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Presentación de solicitudes Fraccionarias (Art 36 D 486)

La presente solicitud corresponde a una divisional de la solicitud parental 13-133020-00000-0000.

4. Objeto de la invención

De acuerdo con lo establecido en el numeral 1.2.2.2 del Capítulo Primero del Título X de la Circular Única para efectos del examen de patentabilidad, se tendrá en cuenta la tasa pagada por el solicitante al momento de comenzar el examen para las reivindicaciones adicionales; esto es que cuando la solicitud contenga más de diez (10) reivindicaciones y no se haya pagado la tasa complementaria, sólo se estudiarán las cubiertas por la tasa pagada. Con relación a la presente solicitud se estudiarán las reivindicaciones 1 a 12 de acuerdo con el radicado 14-274204-00000-0000.

Título de la solicitud: "Combinaciones de principios activos que comprenden piridietilbenzamidas y otros principios activos".

El problema planteado en esta solicitud consiste en la necesidad de suministrar combinaciones que comprendan ingredientes activos útiles para combatir plagas en plantas.

Para resolver este problema técnico la solicitud en estudio proporciona una combinación de fluoripam y *Metarhizium anisopliae*.

El objeto de la presente solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): A01N31/08, 43/28, 57/32; A01P5/00, 7/04.

5. De la solicitud de patente

Título

El título no permite identificar el alcance de la invención ya que define de forma general las combinaciones en términos de su familia química o su actividad biológica, por lo anterior, se sugiere restringir el título incluyendo las características técnicas inventivas. Además, es necesario que el título permita distinguir la presente solicitud de la solicitud original y sus divisionales.

Reivindicaciones (Art 30 D 486, Art 22 PCT)

La reivindicación 1 se encabeza como una combinación entre los compuestos fluopiram y *Metarhizium anisopliae*, sin embargo, de acuerdo con los ejemplos sustentados lo definido corresponde a composiciones, por lo que, se sugiere cambiar el encabezado combinación por composición. Además, las reivindicaciones deben ser claras en cuanto al tipo producto que reclaman.

La reivindicación 2 no es clara porque reclama una procedimiento de preparación sin definirlo en términos de una serie lógica y ordenada de etapas, además, no

establece en estás las condiciones, ingredientes y parámetros que permiten alcanzar el objeto propuesto. Se sugiere hacer las aclaraciones necesarias.

La reivindicación 3 no es clara porque define los excipientes en términos de su función dentro de la composición, se recuerda, que los excipientes se deben definir en términos de su nombre común y proporciones dentro de la composición, se sugiere hacer las aclaraciones necesarias.

Las Reivindicaciones 2-3 incluyen las expresiones “composiciones insecticidas y/o acaricidas y/o nematocidas” y “combatir plagas animales” que refieren el uso de la composición reivindicada, el cual no es una característica técnica que defina a la invención. Se sugiere eliminar dichas expresiones.

La reivindicación 4 no es clara porque la expresión “por ejemplo” precediendo una característica no es limitativa, porque hace opcional a la característica y no precisa el alcance de la reivindicación. Se sugiere incluir una expresión concreta de acuerdo con los ejemplos sustentados.

La reivindicación 10 no es clara porque reclama la inclusión de arcillas modificadas sin caracterizarlas, se sugiere hacer las precesiones necesarias.

Las reivindicaciones 2-12 no son concisas porque las definiciones de los excipientes son generales, por lo que se sugiere restringir la solicitud a una razonable generalización de los excipientes probados, es decir, que se encuentran debidamente sustentados a través de ejemplos concretos de acuerdo con lo presentado en la tabla No 1.

Tabla No. 1 Excipientes de las composiciones soportadas.

Excipientes	Ejemplos probados
Tensioactivos	No sustenta
Diluyentes	No sustenta
Disolventes	Acetona, dimetilformamida
Colorantes	No sustenta
Humectantes	No sustenta
Dispersantes y/o emulsionantes	No define (alquilarilpoliglicoléter)
Antiespumante	No sustenta
Conservante	No sustenta
Espesante	No sustenta
Adhesivos	No sustenta
Gibelinas	No sustenta

Las reivindicaciones 2-12 reclaman que la composición incluye tensioactivos, diluyentes, colorantes, humectantes, antiespumantes, conservantes, espesantes, adhesivos y gibelinas, sin embargo, dentro del capítulo descriptivo no se definen ejemplos concretos de composiciones que comprendan los mencionados excipientes, se aclara, que la mera mención de la posibilidad de incluir unos excipientes seleccionados de una extensa lista no es suficiente soporte, las composiciones se deben definir en términos de sus ingredientes (DCI/IUPAc para los activos y nombre común para los excipientes) y proporciones, por lo anterior, se sugiere eliminar de las mencionadas cláusulas los excipientes no sustentados.

Debido a que la solicitud se refiere a material biológico, es necesario presentar el Certificado de Depósito para la cepa F52 *Metarhizium anisopliae*.

Se requiere al solicitante hacer las aclaraciones necesarias.

6. Determinación del Estado de la Técnica

El estado de la técnica se determinó con base en la fecha de presentación de la solicitud de la cual se invoca prioridad, es decir, diciembre 01 de 2010. Consultadas las fuentes de información con que se cuenta, se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación
D1	US20100249193	"Nematicidal, Insecticidal and Acaricidal Active Ingredient Combinations Comprising Pyridylethylbenzamides and Insecticides"	30/Septiembre/2010
D2	CA2205954	"Pesticides"	26/Enero/2010

El documento D1⁽¹⁾ divulga combinaciones que comprenden fluopiram útiles en el control de plagas producidas por insectos, ácaros y nematodos en suelo, hojas y semillas (Resumen y Pág. 11, Rv. 1).

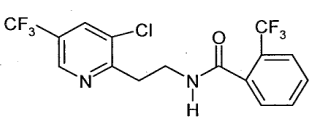
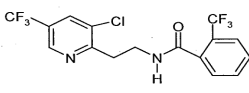
El documento D2⁽²⁾ divulga composiciones que comprenden *Metarhizium anisopliae* combinado con un fungicida útiles en el control de plagas (Resumen y Págs. 24-25, Rvs. 1, 6-7 y 9).

7. Examen de Patentabilidad (Art 14 D486)

Nivel inventivo (Art18 D486)

La reivindicación 1 consiste en "La reivindicación 1 consiste en la "Combinaciones de principios activos que comprenden fluopiram y *Metarhizium anisopliae* y/o cepa F52 de *Metarhizium anisopliae*".

Comparación de las características técnicas esenciales, con las del estado de la técnica:

Características esenciales	D1	D2
Combinación que comprenden:	Combinación de ingredientes que comprende: (Pág. 11, Rv. 1)	Pesticida que comprende: (Pág. 24, Rv. 1)
 (Fluopiram)	 (Fluopiram) (Pág. 11, Rv. 1)	Fungicidas (Pág. 25, Rv. 9)
<i>Metarhizium anisopliae</i> y/o cepa	<i>Metarhizium</i> sp.	<i>Metarhizium anisopliae</i>

1. [www.espacenet/ US20100249193](http://www.espacenet/US20100249193)

2. [www.espacenet/ CA2205954](http://www.espacenet/CA2205954)

F52 de <i>Metarhizium anisopliae</i>	(Pág. 11, Rv. 1)	(Pág. 24, Rvs. 6-7)
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Los documentos D1 y D2 se consideran el estado de la técnica cercano a la invención definida en la reivindicación 1 porque en el caso del documento D1 divulga combinaciones que comprenden fluopiram útiles en el control de plagas producidas por insectos, ácaros y nematodos en suelo, hojas y semillas (Resumen y Pág. 11, Rv. 1). Por su parte, el documento D2 divulga composiciones que comprenden *Metarhizium anisopliae* combinado con un fungicida útiles en el control de plagas (Resumen y Págs. 24-25, Rvs. 1, 6-7 y 9).

La diferencia entre la combinación, definida en la reivindicación 1 y la combinación revelada en el documento D1 consiste en que la combinación reclamada incluye al microorganismos *Metarhizium anisopliae*, mientras que la combinación divulgada en el documento D1 incluye al microorganismo *Metarhizium sp.*

El efecto que logra el utilizar al *Metarhizium anisopliae* en la combinación consiste en que se aumenta la capacidad de combate de la combinación contra plagas.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: “cómo proporcionar combinaciones que comprendan Fluopiram útiles para el control de las infestaciones de plagas en plantas”.

Sin embargo, la combinación de un agente químico fungicida con *Metarhizium anisopliae* útil para tratar infestaciones de plagas en plantas, está divulgada en el documento D2 que enseña composiciones que comprenden *Metarhizium anisopliae* combinado con un fungicida útiles en el control de plagas (Resumen y Págs. 24-25, Rvs. 1, 6-7 y 9).

Además, es parte del conocimiento de la persona normalmente versada en la materia que la cepa F52 de *Metarhizium anisopliae* es aplicada en plantas para prevenir o controlar la infestación de insectos de acuerdo con lo divulgado en los documentos “Effect of Potting Media Components on the Infectivity of *Metarhizium anisopliae* against the Black Vine Weevil (Coleoptera: Curculionidae)”⁽³⁾ y “Field testing of *Metarhizium anisopliae* (strain Met52) for control of cranberry girdler”⁽⁴⁾.

En consecuencia, la persona del oficio normalmente versada en la materia, incluiría dentro de la combinación divulgada en el documento D1 al microorganismos *Metarhizium anisopliae* de acuerdo con lo revelado en el documento D2, para llegar así al objeto de la reivindicación 1 de la solicitud. Por lo cual se considera obvia.

Puesto que las reivindicaciones dependientes 2-12 no mencionan características técnicas esenciales acerca de la combinación, tampoco se pueden considerar inventivas.

3. [http://arsserv0.tamu.edu/SP2UserFiles/person/697/PDF/2008/BruckJEHPottingMediaComponents MS.pdf](http://arsserv0.tamu.edu/SP2UserFiles/person/697/PDF/2008/BruckJEHPottingMediaComponentsMS.pdf)
4. <http://www.bccranberries.com/pdfs/researchreports/Girdler.pdf>

8. Conclusión

Los requisitos de Patentabilidad de la presente solicitud están afectados así:

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	US20100249193	1-12	Nivel inventivo
D2	CA2205954	1-12	Nivel inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir del día siguiente de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante de sesenta (60) días para dar respuesta a este requerimiento y presentarla por escrito.

Finalmente, se le recuerda al solicitante que si como respuesta a este requerimiento llegara a modificar el capítulo reivindicatorio y éste contenga más de diez (10) reivindicaciones, no pagará la tasa de modificación voluntaria pero sí la correspondiente al pago por reivindicación adicional.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.



JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (c)

El anterior requerimiento fue notificado por fijación en lista, de fecha 2015-05-26

Elaboró: German Antonio Clavijo Cohen
Revisó: Jeny Paola Villate Becerra

INFORME DE BÚSQUEDA DE PATENTES PARA EL No de Radicación: 14-274204						
CRITERIOS PARA LA BÚSQUEDA (anexar pantallazo o reporte en PDF, junto con los boleanos utilizados)						
Palabras clave utilizadas		-Fungicidal and <i>Metarhizium anisopliae</i> -Synergistic mixtures comprising <i>fungicidal and Metarhizium anisopliae</i> -Mixture comprising <i>fungicidal and Metarhizium anisopliae</i> -Fungicidal and <i>Metarhizium anisopliae</i> and pesticide mix -Entomopathogenic fungus <i>Metarhizium anisopliae</i> and biocontrol agent <i>metarhizium anisopliae</i> strain F52 and ecology				
CLASIFICACIONES INTERNACIONALES UTILIZADAS PARA LA BÚSQUEDA		A01N31/08, 43/28, 57/32; A01P5/00, 7/04				
BASES DE DATOS CONSULTADAS.						
Google Patents						
WIPO						
Espacenet						
INFORMES DE EXÁMENES REALIZADOS EN OTRAS OFICINAS (documentos patentes, literatura no patente etc...)						
Oficina	Fecha del examen o de reporte de búsqueda de documentos	Anterioridades encontradas para el examen o reportados en el examen de búsqueda(relacionarlos todos)	Reiv. afectadas	RELEVANCIA (requisito que afecta)	FUERON TENIDOS EN CUENTA PARA EL EXAMEN EN LA OFICINA COLOMBIANA	
					SI	NO
EUROPEA	01/06/2013	WO2010108616	1-10	Novedad y Nivel Inventivo	X	
DOCUMENTOS ADICIONALES ENCONTRADOS POR EL EXAMINADOR DE ACUERDO A LOS CRITERIOS DE BÚSQUEDA.						
Effect of Potting Media Components on the Infectivity of <i>Metarhizium anisopliae</i> against the Black Vine Weevil (Coleoptera: Curculionidae)						
Field testing of <i>Metarhizium anisopliae</i> (strain Met52) for control of cranberry girdler						
BÚSQUEDA DE SECUENCIAS						
Número de depósito / patente y N° de secuencia % de identidad						
SEQ ID NO (de la solicitud)	NCBI	The Lens	EMBL-EBI	DDJB	Genome Quest	
	No aplica					

CRITERIOS DE BÚSQUEDA / UNIDAD DE INVENCIÓN (Pantallazos)

No aplica

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

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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/yd³) 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.

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), *Antitrogon parvulus* (Coleoptera: Scarabaeidae) (18) and *Adoryphorus coultoni* (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-

¹Received 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.

²Research Entomologist.

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 ($8.9 \times 8.9 \times 8.9$ cm ($3.5 \times 3.5 \times 3.5$ 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 $\frac{1}{2}$ lb/yd³ 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 ($\pm$ SD) percentage of black vine weevil larvae infected with *M. anisopliae* overall dates among potting media components incorporated with $\frac{1}{2}$ lb/yd³ of formulated *M. anisopliae* granules.

Media component	Mean percentage of infected black vine weevil larvae	
	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 ($\pm$ SD) percentage of black vine weevil larvae infected with *M. anisopliae* among all potting media types incorporated with $\frac{1}{2}$ lb/yd³ of formulated *M. anisopliae* granules at each sample date.

Day	Mean percentage of infected black vine weevil larvae	
	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)

^zSample 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 10⁵ to 10³ 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 ($\pm$ SD) percentage of black vine weevil larvae infected with *M. anisopliae* at each sample date in each potting media component incorporated with $\frac{1}{2}$ lb/yd³ of formulated *M. anisopliae* granules.

Day	Media component	Mean percentage of infected black vine weevil larvae	
		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

^zMeans followed by the same letter on the same sample date within a column are not significantly different ($P < 0.05$) (19).

^ySample 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. Charnley, 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 *Ligurs 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.

Field testing of *Metarhizium anisopliae* (strain Met52) for control of cranberry girdler

Final Report to:
BC Cranberry Growers Association
BC Cranberry Marketing Commission

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Executive Summary:

Cranberry girdler is a difficult pest to control and more options are needed for its management. One possible tool could be the entomopathogenic fungus *Metarhizium anisopliae*, which has shown some promise for girdler control in previous studies conducted in Washington. A field study using microplots was conducted to determine the efficacy of an emulsifiable concentrate formulation of *M. anisopliae* strain F52 (Met52). Cranberry microplots were treated with Met52 at two time intervals - one week prior to egg infestation and at egg infestation and at two different rates - 1X (12 L/Ha) and 2X (24 L/Ha), for a total of four different Met52 treatments. Plots for these four treatments and the untreated Control were infested with cranberry girdler eggs, which were obtained from moths collected from commercial cranberry fields during late June and early July. Microplots were placed out in research cranberry beds to expose larvae and the fungus to environmental conditions similar to a commercial field. Although fewer larvae were recovered from the 2X treatments and the treatments where Met52 was applied one week prior to egg infestation than in untreated Control - these differences were not statistically significant. We examine the combined evidence for *M. anisopliae* against both cranberry girdler and black vine weevil and suggest that while it is still a potential tool for cranberries, the experimental work needed to obtain robust efficacy data to support a product registration could be substantial.

Introduction: Cranberry girdler (*Chrysoteuchia topiaria*) is a difficult to control pest of cranberries in BC. Below ground feeding damage by girdler larvae can result in areas of poor growth or possibly vine death. There are no insecticides registered for girdler control in cranberries in Canada (AAFC 2007) and BC growers are using a combination of cultural control and entomopathogenic nematodes for management of this pest. Although nematodes can effectively control girdler larvae if used correctly, application timing and maintenance of optimal field conditions pre and post application can be challenging. Another control tool, the entomopathogenic fungus *Metarhizium anisopliae* may be a good candidate for the pest management tool box in cranberries. Various strains and formulations of *M. anisopliae* have been shown to control black vine weevil and other soil dwelling insect pests (Ansari *et al.* 2010, 2009, Fisher and Bruck 2008, Bruck *et al.* 2005), the fungus appears to remain active in the soil for several months after application (Bruck 2005, Booth *et al.* 2000) and it is considered a reduced-risk product with some formulations approved for organic agriculture. In terms of *M. anisopliae* efficacy against cranberry girdler the results have been promising, but not conclusive.

Small field-plot assays to test the efficacy of a local (to Washington) strain of *M. anisopliae* against cranberry girdler demonstrated significant reduction in adults (Booth and Shanks 1998) and a non-significant reduction in larvae (Booth *et al.* 2000) compared to Control plots. Similarly, in our previous work there was a trend towards fewer feeding tunnels in roots and fewer larvae overall in Met52 treatments than Control, however this result was also not significant (Prasad and Soto, 2009 Project Report to Cranberry Commission). Met52 is a commercially available strain of *M. anisopliae* that is registered in Canada for black vine weevil control in containerized nursery stock. In preliminary work, we found that directly sprinkling Met52 granules (60kg/ha) on to cranberry foliage, caused a non-significant reduction in survival of weevil larvae in experimental cranberry beds (E.S. Cropconsult unpublished data; Fig. 1). In our 2009 trials with girdler we also directly sprinkled Met52 granules onto the soil surface, as was the protocol for Booth *et al.* (2000). So one challenge of effectively using Met52, or other *M. anisopliae* strain, in cranberries may be to effectively deliver the product (fungal spores) to the area where the pest can become exposed. Recently a liquid formulation of Met52, which could be suitable for delivery via chemigation, has been developed. The objective of this trial was to continue to evaluate the efficacy of *Metarhizium anisopliae* strain Met52 for control of cranberry girdler. If effective such a tool would be useful to both organic and conventional cranberry production.

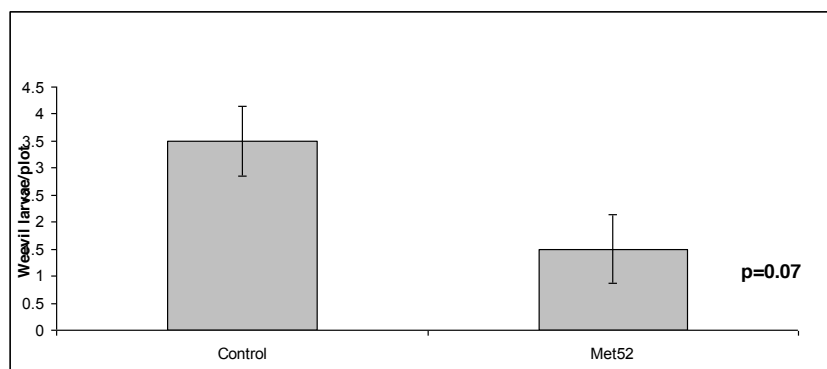


Figure 1. Preliminary trial results of Met52 efficacy against black vine weevil in cranberries. Trial conducted in small experimental beds. Bars represent the mean ($\pm$ s.e.) of 4 replicates/treatment.

Methods:

Moth and egg collection - Cranberry girdler adults were collected from commercial cranberry fields in Pitt Meadows, Langley and Richmond, BC from June 26 to July 13, 2010. Moths were collected directly from foliage and into 100 ml vials (1 moth per vial). Initially moths were placed inside a rearing cage with a sugar and water solution to promote egg laying (Fitzpatrick et al. 2001). However, we noticed that many of the moths collected on later dates (June 29 and onwards), laid eggs within a day of capture directly into the vials. Thus we modified our egg collection approach - leaving moths inside their vials for 24 to 48 h during which time eggs were laid. A major advantage of this approach for collecting eggs is that we avoided overstocking the rearing cage with males and thus having poor egg production in rearing cages. Vials where the majority of eggs turned orange were then placed in the fridge (most females by this time had died and could be easily removed). This was another advantage of using the vials for oviposition - less handling of eggs to separate fertilized (orange) and unfertilized (white) eggs from each other. The eggs used for the trial were from three different batches of moths collected on 1) June 30 to July 1, 2) July 7 to July 8 and 3) July 12 to July 14. These eggs were placed in the fridge for 24 to 72-h prior to being used in the trial.

Preparation of test plots and infestation with girdler eggs - For this trial we had two types of test plots - cranberry microplots (Fig. 2a) and 1 m X 1m "field" plots that were inside our research cranberry beds (Fig. 2b). All of the plots were located outdoors in Abbotsford, BC and exposed to ambient environmental conditions. Microplots consisted of 9 cm diameter (0.64 m²) plastic cup planted with cranberry plugs (roots and shoots) and filled with potting media (Sunshine Mix 1). To increase the root material available for girdler larvae to feed on we also sprinkled 2 grams of reed canary grass seeds on the surface of the soil. Cranberry plugs were planted into cups 21 days prior to the start of the trial and grass seeds added 2 days prior. The soil in microplots was watered so that the soil was moist, but not saturated, prior to the introduction of eggs. There were no drainage holes in the cups. Subsequently, microplots were irrigated carefully to keep soil evenly moist but standing water was avoided in order to prevent girdler larvae from drowning (Fitzpatrick 2007).

Research cranberry (var: Stevens) beds were four years old at the time of the trial. Vines were obtained from a commercial cranberry farm. No girdler damage has been observed in these beds since they were planted. The individual field plots were marked off with flagging tape and the area where eggs were added to plots was marked with a flag. We selected an area, within each field plot, where vine establishment was healthy and upright growth was lush. Field plots were irrigated for two hours prior to the start of the trial and then for approximately 30 minutes every two to four days after depending on weather and rainfall.



Figure 2. A (Left): Microplots - cranberries were planted in cups 3 weeks prior to start of trial. After treatment and egg infestations cups were dug into research cranberry beds. B (Right): "Field" plots placed inside research cranberry beds. Cranberry girdler eggs were sprinkled in the area around flags.

Girdler eggs were gently sprinkled over the entire soil surface of microplots or within a 10-cm diameter area around each flag in field plots (Fig. 2b). We used 90 eggs/microplot and 75 eggs/field plot. We used such a large number of eggs for infestation, even for our relatively small microplots, because our plots were open to other arthropods and we were concerned that egg predation from rove beetles (Haase-Statz 1997) and other predators like centipedes (Mahr 2005) could occur. Previously we conducted this trial in a greenhouse to avoid the predator problem, but even in a greenhouse beetles and centipedes managed to infest pots (R. Prasad and M. Soto, personal observations, September 2009).

Girdler eggs were handled with a fine paint brush, counted out under a microscope and transferred to wax paper envelopes from which they were sprinkled. Thus the physical handling of eggs was kept to a minimum. There were two rounds of egg infestation, based on treatment (see below), for microplots the first and second rounds of egg infestation occurred on July 5 and July 11. In the field plots, the first and second rounds of egg infestation occurred on July 10 and July 16.

Application of treatments - Originally our work plan was to test the granular formulation of *Metarhizium anisopliae* Met 52 (Novozymes Biologicals Inc., Salem VA) at two rates: 60 kg/ha (used by Booth et al. 2000) and 120 kg/ha. However in discussion with Novozymes a newer emulsifiable concentrate (EC) formulation of Met52 was recommended for the trial (J. Leland, Novozymes Biologicals, Inc. personal communication June 2010). Met52 EC is five times more concentrated than the granular formulation and the rates recommended for this trial were 12L/Ha and 24L/Ha. For the lower (1X rate) this worked out to be 0.76 ml of product in 50 ml of water for the microplots (0.64 m²) and 1.2 ml of product in 3 L of water for the field plots (1 m²). For the higher (2X rate) this worked out to be 1.5 ml of product in 50 ml of water for the microplots and 2.4 ml of product in 3 L of water for the field plots. For Control microplots only 50 ml of water was added and 3 L of water was added to Control field plots.

Treatments were applied at two different time intervals - on the same day that eggs were added to plots and one week before eggs were added to plots. Microplots were all treated with Met 52 on July 5. Thus the trial consisted of four combinations of rate X application timing and a Control treatment for a total of five treatments:

- 1) 1X rate - at egg infestation (eggs added July 5 (microplots) & July 10 (field))
- 2) 1X rate - one week before egg infestation (eggs added July 11 (microplots) & July 16 (field))
- 3) 2X rate - at egg infestation (eggs added July 5 (microplots) & July 10 (field))
- 4) 2X rate - one week before egg infestation (eggs added July 11 (microplots) & July 16 (field))
- 5) Untreated control (eggs added July 5 microplots July 10 (field))

Each treatment was replicated six times for a total of 30 microplots and 30 field plots. After treatment microplots were planted into field plots with the same treatment regime. Cups were buried so that 3 cm of the top of the cup was above the ground. This ensured that microplots were exposed to the same environmental conditions of the field plots but hopefully minimized predator movement into cups. Field plots were treated on July 10.

Assessment - Microplots were assessed on August 31, 8 weeks after they were infested with girdler eggs. Plots were assessed by turning over cup contents and sifting through soil and roots to find frass (Fig. 3) and larvae. Field plots were assessed on September 6, when larvae are still expected to be active and 8 weeks from when eggs were added to plots. Plots were assessed by removing a 40 cm x 40 cm x 5 cm deep patch of soil around the area where eggs were released. These soil samples were then sifted through by hand and signs of frass, root feeding or girdler larvae were recorded.



Figure 3. Cranberry girdler frass was easily detected from the potting media and plant roots in microplots.

Analysis of data - Only microplot data were analyzed as only one girdler larva was recovered from field plots. The impact of the four different Met52 treatments on the recovery of girdler larvae was analyzed using one-way ANOVA. Since frass was also recovered from microplots we also analyzed these data, in combination with larval

counts, using a 5 X 2 contingency table with presence/absences of frass + live larva(e) as our two levels of response variable and treatments as five levels of the X-variable. Data were analyzed using JMP-IN (Version 5.1, SAS Institute Chicago, IL). All raw data from microplot trials are presented in Appendix 1.

Direct mortality bioassay (preliminary trial) - The four larvae recovered from the Control plots were used for a very preliminary Petri dish bioassay to determine if Met52 can cause direct mortality to girdler larvae. We followed the soil bioassay protocol provided by Novozymes Biologicals, but at a much smaller scale and only at a single rate (the highest). To 100 g of sterile moist soil we added 1 mL of Met52EC. The soil was then mixed and placed in a container and a single girdler larva was added. This protocol was repeated twice for a total of two larvae in two dishes with Met52. For the Control treatment a single larva was placed in a container with 100g of moist sterile soil. There were two replicates of the Control treatment. All four containers were sealed with tape and placed individually in Ziploc bags to minimize movement of spores. Containers were kept at under low light conditions at $19 \pm 1^\circ\text{C}$ and were checked after 7 and 10 days. Soil was checked every other day but remained moist throughout the trial so no additional water was required. All larvae used for the bioassay were obtained from the Control microplots - thus had no prior exposure to Met52 and were approximately 1 cm to 1.5 cm in length at the time of the trial.

Results and Discussion: Overall the number of larvae recovered was quite low and there were no significant treatment effects on the number of live larvae recovered from microplots (Fig. 4; $F(4,25) = 1.41$, $p = 0.26$). When we examined the combined response of frass + larvae we still did not see a significant effect of Met52 treatment on reducing cranberry girdler activity (Table 1; Likelihood Ratio $\chi^2 = 8.21$, $df = 4$, $p = 0.084$). Although not significant the overall trend is for fewer larvae recovered in microplots treated with Met52 either prior to egg infestation or at 2X the rate compared to the untreated Control. Further, in our preliminary bioassay we saw indications of *M. anisopliae* infection on the two larvae exposed to Met52 in the soil bioassay - both larvae were dead after 7 days, and covered with fuzzy grey-green fungal growths (Fig. 5). In contrast the two Control larvae were either still alive or dead but not covered with any fungal growths.

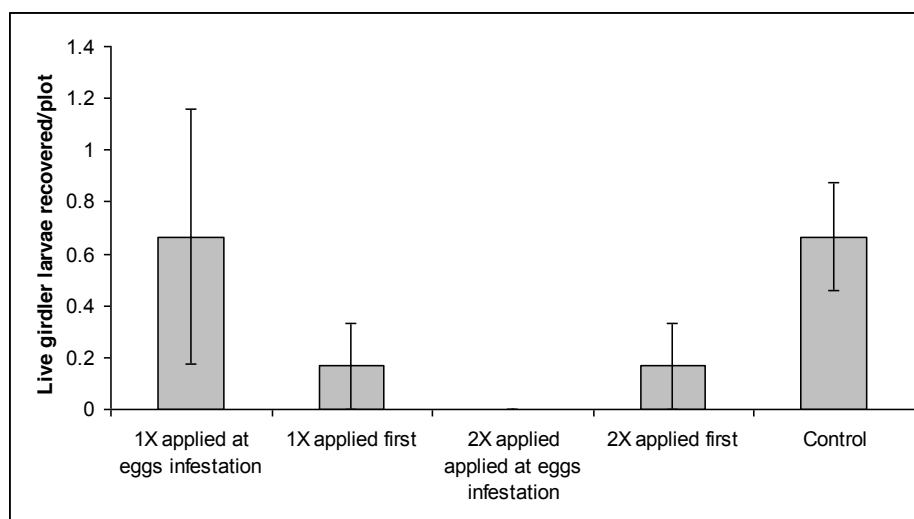


Figure 4. Effect of rate and application timing of *Metarhizium anisopliae* (strain F52) on the recovery of cranberry girdler (*Chrysoteuchia topiaria*) larvae from cranberry microplots. Bars represent the mean $\pm$ s.e. of 6 replicates/treatment (total N = 30).

Table 1. Effect of rate and application timing of *Metarhizium anisopliae* (strain F52) on the recovery of cranberry girdler (*Chrysoteuchia topiaria*) larvae and/or frass from cranberry microplots.

Treatment	Proportion of pots with frass + larvae (#pots out of 6)	Proportion of pots with at least one live larvae (#pots out of 6)	Average number of larvae recovered
Control (eggs only)	0.83 (5/6)	0.67 (4/6)	0.67 (4 live larvae total)
1X Met52 applied before eggs	0.17 (1/6)	0.17 (1/6)	0.17 (1 live larvae total)
1X Met52 applied on same day as eggs	0.33 (2/6)	0.33 (2/6)	0.67 (4 live larvae total)
2X Met52 applied before eggs	0.67 (4/6)	0.17 (1/6)	0.17 (1 live larvae total)
2X Met52 applied on same day as eggs	0.17 (1/6)	0	0

In our 2009 trials, conducted with the granular formulation of Met52, we also saw a similar trend towards fewer cranberry girdler larvae in Met52 treated pots than the untreated Control, but again this trend was not statistically significant. Finally, in preliminary trials conducted in research cranberry beds, we observed a similar non-significant trend towards fewer black vine weevil larvae recovered from Met52 granular treated plots than untreated Control (E.S. Cropconsult Ltd. unpublished data). Our hope was that the EC formulation of Met52 would be better at penetrating to the root zone of cranberries and we would see better control of cranberry girdler. Although there are

several reports confirming the efficacy of granular formulation of Met52 against black vine weevil (Bruck and Donahue 2007, Bruck 2005), since the original work of Booth and Shanks (1998) and Booth et al. 2000 there have been no published reports, to our knowledge, examining the impact of *M. anisopliae* on cranberry girdler. This is surprising since cranberry girdler is also an important pest of sod and turf (Booth and Shanks 1998) and earlier crop profiles for cranberries have Metarhizium for girdler control listed as research priorities (e.g. Wisconsin 1998 <http://www.ipmcenters.org/cropprofiles/docs/wicranberries.pdf> and New Jersey 1997 <http://pestmanagement.rutgers.edu/njinpas/CropProfiles/cranberryprofile.pdf>).



Figure 5. Dead cranberry girdler larvae (above arrow) from Met52 soil bioassay.

In their original work Booth and Shanks (1998) observed a significant reduction in emergence of male girdler moths from plots treated the summer previous with a very high rate of a local strain of *M. anisopliae* (200g of granules/m²). In follow up work (Booth *et al.* 2000) conducted with an improved application process for *M. anisopliae*, a lower and more affordable rate of granule application (6 g/m²) was used. At one field site there were significantly fewer larvae in the *M. anisopliae* treated plots than the Control (see Table 4, in Booth *et al.* 2000). But in the remaining three of the four field sites tested there were no significant differences in the number of larvae between treated and Control plots - but like our results some sites did have fewer larvae in *M. anisopliae* treated plots than Control. Thus the previous work by E.S. Cropconsult and others provides indications that Met52 or other strains of *M. anisopliae* may be effective against cranberry girdler but the results have not been robust. Application timing, rate, and delivery of product to the target area may all be factors that could be causing the inconsistent results with *M. anisopliae* for girdler control. Cranberry girdler is a challenging pest to work with (see below) and this may be another reason why very few published studies have followed up on the findings of Booth and Shanks (1998).

Suggestions for Next Steps: While the results of the past two studies have not been conclusive, we suggest that trial work with *M. anisopliae* not be abandoned. The granular formulation of Met52 does have demonstrated efficacy against black vine weevil (Bruck and Donahue 2007) and the preliminary results of the soil bioassay from this study suggest that there is direct mortality to cranberry girdler larvae. Thus *M. anisopliae* (Met52 or other formulations) have the potential to provide control of two cranberry pests. The advantage of Met52 is that the granular formulation is already registered for (non-food) use in Canada and the EC formulation could be suitable for chemigation.

- Repeat at a larger scale the soil bioassay for girdler to confirm that cranberry girdler is susceptible to Met52 EC formulation. If soil bioassays are conclusive and promising *then* consider field trials (microplot, field plot or other) for cranberry girdler.
- Field trials to determine efficacy of Met52 EC formulation against black vine weevil in cranberry fields (Issues to consider are timing of application, rate of EC formulation, and water volumes accompanying application).
- However, there are some major challenges for any future trials with girdler (and weevil). These challenges include
 - producing a sufficient amount of eggs to adequately infest plots. Although an individual female can lay many eggs (reportedly 300 on the first day (Mahr 2005)) we have found it challenging to obtain this many eggs from a single female or to infest pots/plots at a level that yields more than 4 larvae. Egg predation may be a big factor in trials.
 - rearing a sufficient number of larvae for bioassays
 - conducting the trial in conditions that are representative of a typical cranberry field
 - obtaining a site large enough to have a sufficient number of replicates and permission to introduce either or both pest

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Literature cited:

Agriculture and Agri-Food Canada (AAFC). 2007. Crop profile for cranberry in Canada. 57 pp.

Ansari, M.A., Shah, F.A. and Butt, T.A. 2010. The entomopathogenic nematode *Steinernema kraussei* and *Metarhizium anisopliae* work synergistically in controlling overwintering larvae of the black vine weevil *Otiorhynchus sulcatus*, in strawberry growbags. *Biocontrol Science and Technology* 20(1): 99-105.

- Ansari, M.A., Evans, M., and Butt, T.A. 2009. Identification of pathogenic strains of entomopathogenic nematodes and fungi for wireworm control. *Crop Protection* 28(3): 269-272.
- Booth, S.R. and Shanks C.H. 1998. Potential of dried rice/mycelium formulation of entomopathogenic fungi to suppress subterranean pests in small fruits. *Biocontrol Science and Technology* 8(2): 197-206.
- Booth, S.R., Tanigoshi, L. and Dewes, I. 2000. Potential of dried mycelium formulation of an indigenous strain of *Metarhizium anisopliae* against subterranean pests of cranberry. *Biocontrol Science and Technology* 10:659-668
- Bruck, D.J. and Donahue, K.M. 2007. Persistence of *Metarhizium anisopliae* incorporated into soilless potting media for control of the black vine weevil, *Otiorhynchus sulcatus* in container-grown ornamentals. *Journal of Invertebrate Pathology* 95: 146-150.
- Bruck, D.J. 2005. Ecology of *Metarhizium anisopliae* in soilless potting media and the rhizosphere: Implications for pest management. *Biological Control* 32:155-163
- Bruck, D.J., Snelling, J.E., Dreves, A.J. and Jaronski, S.T. 2005. Laboratory bioassays of entomopathogenic fungi for control of *Delia radicum* (L.) larvae. *Journal of Invertebrate Pathology* 89: 179-183.
- Fisher, J.R. and Bruck, D.J. 2008. Biology and control of root weevils on berry and nursery crops in Oregon. *Acta Horticulturae* 777: 345-351.
- Fitzpatrick, S.M. 2007. Survival of submerged larvae of cranberry girdler, *Chrysoteuchia topiaria*, in the laboratory. *Crop Protection* 26(2007):1810-1816
- Fitzpatrick, S.M., Newhouse, J.A., Troubridge, J.T. and Weitemeyer, K.A. 2001. Rearing the cranberry girdler *Chrysoteuchia topiaria* (Lepidoptera: Pyralidae) on reed canary grass *Phalaris arundinacea* (Festucoideae: Panicoideae). *Journal of the Entomological Society of British Columbia* 98: 211-215.
- Haase-Statz, S. 1997. Evaluation of *Paederus littorarius* (Coleoptera : Staphylinidae) as an egg predator of *Chrysoteuchia topiaria* (Lepidoptera : Pyralidae) in Wisconsin cranberry bogs. *Great Lakes Entomologist* 30(3): 93-98 (Abstract only)
- Mahr, D. 2005. Cranberry girdler. Fact sheet. http://www.hort.wisc.edu/cran/mgt_articles/articles_pest_mgt/insects/profiles_insects/GIRDLER.pdf. First access May 2009.

Appendix 1 - Raw data from microplots (note that only 1 larvae was recovered from field plots thus raw data are not presented for that portion of the trial).

Treatment & Replicate	Frass present	# of cranberry girdler larvae	<i>Other</i>
Control 1	Present	0	
Control 2	Present	1	
Control 3	Present	1	
Control 4	Present	1	
Control 5	Absent	0	
Control 6	Present	1	
1X - at infestation 1	Present	1	<i>2 cutworm larvae</i>
1X - at infestation 2	Present	3	
1X - at infestation 3	Absent	0	<i>1 cutworm larva</i>
1X - at infestation 4	Absent	0	
1X - at infestation 5	Absent	0	
1X - at infestation 6	Absent	0	
2X - at infestation 1	Absent	0	
2X - at infestation 2	Absent	0	
2X - at infestation 3	Absent	0	
2X - at infestation 4	Present	0	
2X - at infestation 5	Absent	0	
2X - at infestation 6	Absent	0	
1X - 1 week pre 1	Absent	0	
1X - 1 week pre 2	Absent	0	
1X - 1 week pre 3	Present	1	<i>1 cutworm larva</i>
1X - 1 week pre 4	Absent	0	
1X - 1 week pre 5	Absent	0	
1X - 1 week pre 6	Absent	0	
2X - 1 week pre 1	Present	0	
2X - 1 week pre 2	Present	1	
2X - 1 week pre 3	Present	0	
2X - 1 week pre 4	Absent	0	<i>1 cutworm larva</i>
2X - 1 week pre 5	Absent	0	<i>1 cutworm larva</i>
2X - 1 week pre 6	<i>Present</i>	<i>0</i>	<i>1 cutworm larva</i>