Diversity of rhizosphere associated entomopathogenic fungi of perennial herbs, shrubs and coniferous trees

Joanna J. Fisher[a], Stephen A. Rehner[b], Denny J. Bruck[c,*]

[a] Oregon State University, Bioresource Research Program, Corvallis, OR 97330, United States
[b] USDA-ARS Systematic Mycology and Microbiology Laboratory, Beltsville, MD 20705, United States
[c] USDA-ARS Horticultural Crops Research Laboratory, 2420 NW Orchard Avenue, Corvallis, OR 97330, United States

ABSTRACT

Understanding habitat selection of fungal entomopathogens is critical to improve the efficacy, persistence and cost of these fungi as microbial insecticides. This study sought to determine the prevalence of Metarhizium and Beauveria spp. isolated from the rhizosphere of strawberry, blueberry, grape and Christmas tree crops in the Willamette Valley of Oregon. Entomopathogenic fungi were assigned to thirteen species based on molecular phylogenetic criteria. Four species of Metarhizium were isolated including Metarhizium brunneum, Metarhizium guizhouense, Metarhizium robertsi and Metarhizium flavoviride var. pemphigii. Nine Beauveria species were isolated including Beauveria brongniartii, an undescribed species referred to as Clade C and seven phylogenetic species of Beauveria bassiana. Strawberries and blueberries were significantly associated with M. brunneum and Christmas trees with M. guizhouense and M. robertsi. Grapes were significantly associated with B. bassiana phylogenetic species Bbas-16. All of the Metarhizium isolates screened were pathogenic to Otiorhynchus sulcatus larvae in laboratory bioassays but only M. brunneum and M. robertsi caused significant levels of infection. The study results suggest that certain species of Metarhizium and Beauveria are significantly associated with the strawberry, blueberry and Christmas tree rhizosphere and could potentially provide better control of O. sulcatus.

1. Introduction

Key to promoting epizootic development of entomopathogenic fungi is a thorough knowledge of their ecology and life history. The ecology and life history of entomopathogens can vary considerably among species (Pell et al., 2010); therefore it is imperative to have a reliable method of species identification. In the case of both Metarhizium and Beauveria, morphological cryptis occurs between both sister and non-sister taxa, probably as a result of heterogeneous morphological evolution, retention of symplesiomorphic morphologies and convergent morphological evolution due to occupation of similar ecological niches. As a result of these confounding evolutionary processes, morphological identification is not sufficient for distinguishing between species (Bischoff et al., 2009). With recent genetic analysis, it is now possible to identify members of both Metarhizium and Beauveria spp. (Bischoff et al., 2009; Rehner and Buckley, 2005; Rehner et al., 2006; Rehner, unpublished data).

According to Vega et al. (2009), a key question that needs to be addressed in the use of entomopathogenic fungi in insect microbial control is to determine the extent to which species engage in unique associations with host plants. Thus, future research should integrate efforts to understand the capacity and significance of entomopathogenic fungi’s role as endophytes, plant disease antagonists, plant growth promoters and rhizosphere colonizers in addition to their pathogenicity and virulence toward their intended insect hosts.

Metarhizium anisopliae (Metschnikowia) Sorokin (Hypocreales: Clavicipitaceae) has been commercially developed for the potted nursery industry as a granular formulation (FS2, Novozymes Biologicals Inc., Salem VA, USA), which is incorporated into growing media at potting for Otiorhynchus sulcatus control (Bruck and Donahue, 2007). Beauveria bassiana (Balsamo-Crivelli) Vuillemin (Hypocreales: Cordycipitaceae) (GHA, BotaniGard ES™ Laverlam International Corporation, Butte, MT; ATCC 4040, Naturalis, Troy Biosciences Inc., Phoenix, AZ) is also commercially available and primarily used by growers as a foliar spray for the control of greenhouse pests. Despite their ability to kill insects, entomopathogenic fungi applied inundatively have performed inconsistently, due in large part to a lack of understanding of their ecology and biology and the expectation that they will perform similarly to synthetic pesticides (Roy et al., 2010). Historically, entomopathogens have been selected for release in the field based solely on their efficacy in laboratory bioassay tests, without consideration of their micro-habitat preferences and ecological constraints. It has been assumed...
that fungal population genetics are closely related to host insects (Bidochka et al., 2001). However, recent research shows that *M. anisopliae* population structure may be driven by habitat selection, not insect host selection (Bidochka et al., 2001). Similarly, *B. bassiana* has adapted to selected habitats and any evidence of an in-host-related population structure should be viewed primarily as coincidental and not as a result of co-evolution (Bidochka et al., 2002; Meyling and Eileenberg, 2006, 2007; Meyling et al., 2009). In temperate North American boreal forests, *B. bassiana* is more abundant in natural habitats while *M. anisopliae* is more abundant in agricultural habitats (Bidochka et al., 1998). A number of different factors have been studied in terms of their effect on the distribution of entomopathogenic fungi in the soil, including geographical location, habitat type, soil type and soil tillage (Rath et al., 1992; Sosa-Gomez and Moscardi, 1994; Vanninen, 1996). In addition to the important role that large landscape-scale habitat selection plays in the abundance and distribution of entomopathogenic fungi, it is becoming increasingly apparent that consideration of the microhabitats that entomopathogenic fungi occupy also plays an essential role when developing screening strategies for selecting strains as microbial control agents. *B. bassiana* was isolated from the wheat rhizosphere in Australia (Sivasithamparam et al., 1987), but the potential implications of this discovery on the microbial control of insects was not appreciated at the time. *M. anisopliae* has more recently been found in the cabbage rhizosphere; however, again the pest management implications of this phenomenon were not explored (Hu and St. Leger, 2002). The population of *M. anisopliae* in the inner cabbage rhizosphere remained at $10^5$ propagules/g, while the populations in the non-rhizosphere soil decreased from $10^7$ to $10^6$ propagules/g after several months. Hu and St. Leger (2002) also noted that the carrying capacity of *M. anisopliae* (2575-GFP) in the cabbage rhizosphere ($10^6$ propagules/g) was higher than the LC50 value of the isolate against a number of insect pests. The pest management potential of rhizosphere colonization by entomopathogenic fungi was not determined directly until a study by Bruck (2005). Colonization of the *Picea abies* (L.) Karst. (Pinaceae: Pinaceae) rhizosphere by *M. anisopliae* (FS2) provided nearly 80% control of *O. sulcatus* larvae after 2 weeks of exposure to inoculated roots (Bruck, 2005). Rhizosphere colonization can also directly affect the efficacy of the fungi as a microbial control and can contribute largely to its success or failure by modifying the behavioral response of an insect host to its fungal entomopathogen. *O. sulcatus* larvae are more attracted to *P. abies* plants than to *Picea engelmannii* (M. of *P. abies* plants grown in the absence of fungal spores (Kepler and Bruck, 2006).

The black vine weevil, *O. sulcatus* (Coleoptera: Curculionidae), is a parthenogenic, polyphagous insect pest commonly found infesting container and field-grown ornamentals and small fruits throughout the Pacific Northwest. *O. sulcatus* has a host range of over 150 plant species (Moorhouse et al., 1992), primarily in the families Ericaceae, Pinaceae, Primulaceae, Rosaceae, Saxifragaceae, Taxaceae and Vitaceae (Cowles, 1995). *O. sulcatus* originated in Northern Europe and now occurs throughout the major nursery and small fruit growing regions of the United States, Canada, Australia, Japan, Chile and New Zealand. Movement throughout the world is associated with shipments of contaminated plants (Moorhouse et al., 1992). A wide host range coupled with a cryptic life cycle makes this insect a formidable pest of the nursery and small fruit industries.

Chemical, cultural, and biological controls can be used to manage *O. sulcatus*. Chemical controls are most effective in managing adult *O. sulcatus* if several applications are made to target preovipositional adults at night when the weevils are active (Moorhouse et al., 1992; Son and Lewis, 2005). The larval stage can be targeted with fall or spring soil drenches or by incorporating a pesticide into the growing media prior to potting. High volume sprays or heavy irrigation after pesticide application is required for effective penetration of larvae drenched on the soil surface (Moorhouse et al., 1992). Cultural control practices such as crop rotation; early season plowing and use of cover crops that are unattractive to weevils can slow the spread of infestation and reduce weevil populations but are not effective eradication methods (Moorhouse et al., 1992). Entomopathogenic nematodes can effectively control *O. sulcatus* larvae when applied in spring or fall (Bruck, 2004a) but their adoption has been limited due to their cost, short shelf life, unpredictable performance and low persistence (Georgis et al., 2006). Improved knowledge of the biology of rhizosphere associated fungi will allow for the development of novel control methods for root-feeding pests, including *O. sulcatus*.

In this study, the principal objective was to ascertain if there was an association between host plant and species of fungi by using phylogenetic analysis to determine the prevalence of naturally-occurring entomopathogenic fungi in the rhizosphere of *O. sulcatus* susceptible crops grown in the Willamette Valley of Oregon. The second objective of this study was to access the pathogenicity of the *Metarhizium* spp. isolated to this key root-feeding insect.

### 2. Material and methods

#### 2.1. Collection of root samples

Root samples were collected from strawberry, *Fragaria* (L.) (Rosales: Rosaceae), blueberry, *Vaccinium* (L.) (Ericales: Ericaceae), grape *Vitis* (L.) (Rhamnales: Vitaceae) and the following Christmas tree species: *engelmann spruce, Picea engelmannii* (Parry) ex engelm (Pinaceae: Pinaceae), noble fir, *Abies procera* (Rehder) (Pinaceae: Pinaceae) and douglas fir, *Pseudotsuga menziesii* (Mirth) franco (Pinaceae: Pinaceae). Root samples were collected from strawberry and blueberry fields using a standard golf hole core (10.2 ± 17.8 cm, Pro II Hole Cutter, Markers Inc., Avon Lake, OH). Christmas tree and grape samples were collected using a shove due to the difficulties associated with using a golf hole core in the dry soils in those fields. Fields containing established plantings (>3 and 10 yrs for strawberries and other plants (blueberries, grapes, Christmas trees), respectively) were preferentially sampled over fields with younger plants so as to allow time for any entomopathogenic fungi present in the soil to colonize the rhizosphere. Root samples were randomly collected from plants distributed throughout the field, or, if the field was over five hectares, samples were taken from a five hectare section within the field. Roots were placed in 3.78 l plastic reclosable bags, placed in a cooler with ice until they were returned to the laboratory and refrigerated until use (0–4 days). A total of 7, 11, 6 and 10 strawberry, blueberry, Christmas tree and grape fields were sampled throughout the Willamette Valley of Oregon yielding collections of 70, 109, 60 and 100 root samples from each crop, respectively (refer to Table 1 for a list of field locations).

#### 2.2. Fungal isolation and culturing

Entomopathogenic fungi were isolated from the rhizosphere of all plants using a modified version of the 'Galleria bait method' (Zimmermann, 1986). Individual roots were shaken in a standardized manner to remove non-rhizosphere soil and then placed into a deep dish Petri plate (150 × 25 mm, Thermo Scientific, Waltham, MA) that contained moistened filter paper (15 cm, Grade p5, Fisher, Pittsburgh, PA). The rhizosphere soil was defined as the soil still adhering to the roots after shaking. Live wax worms, *Galleria mellonella* (L) (Lepidoptera: Pyralidae) were placed in the Petri plates and allowed to crawl and contact the roots and the rhizosphere soil. The Petri plates were sealed with Parafilm and placed in plastic reclosable bag and incubated in complete darkness at

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21 °C. After 2 weeks, dead larvae were removed and placed in small snap-lock Petri dishes (9 × 50 mm; Becton Dickinson, Franklin Lakes, NJ) containing moistened filter paper (Whatman #1) and incubated in complete darkness at 21 °C until sporulation (cadavers that did not sporulate were discarded). After sporulation, fungi were isolated and cryo-preserved using the procedures outlined by Bruck (2004b). The prevalence of each fungal species/clade in the rhizosphere of each host plant was analyzed using a Fisher’s exact test (P < 0.05) (SAS Institute, 1999).

Single-spore subcultures were established from a single infected G. mellonella larva per root sample. If a sample contained larval tissue, fungal DNA was extracted. Mycelia were collected by centrifugation, washed 3× in sterile distilled water and excess moisture removed by blotting the mycelia between two layers of sterile filter paper. The mycelia were lyophilized overnight (Free Zone6, Labconco, Kansas City, MO) and stored at −20 C.

2.3. DNA extraction

Approximately 25–50 mg of lyophilized mycelium was pulverized with glass-zirconia beads (Biospec, Bartlesville, OK) in a Fastprep (Q-Biogene, Solon, OH) sample grinder for 6 s at a speed setting of 4.5. The ground tissues were suspended in 500 μl sterile distilled water and excess moisture removed by blotting the mycelia between two layers of sterile filter paper. The mycelia were lyophilized overnight (Free Zone6, Labconco, Kansas City, MO) and stored at −20 C.

2.4. PCR

Metarhizium and Beauveria strains were sequence-characterized with nuclear loci including the 5′ region of elongation factor 1 alpha (5′-tef1) and Bloc, which are informative for diagnosis of phylogenetic species in these genera (Bischoff et al., 2006, 2009; Rehner et al., 2006), respectively. The 5′-tef1 region in Metarhizium, which contains three sequence polymorphic introns, was amplified with primers EF1T (5′-ATGGTAAAGG/A/G/GACAGAC) and EF2T (5′-GGA/G/A/GTTACCTG/C/ATCATGTT) (Rehner and Buckley, 2005). The marker Bloc is an intergenic region developed specifically for Beauveria and was amplified with primers B5.1F (5′-CGACCGGCGCCAATCTTGTA) and B3.1R (5′-GTCTTCCAGTA CCACGC) (Rehner et al., 2006). PCR reactions for each locus included 1 μl genomic DNA extract, 200 μM dNTPs, 0.4 μM each of two locus-specific primers (Integrated DNA Technologies, Coralville, IA), and 1.0 unit of Taq DNA polymerase (Promega, Madison, WI) in a total reaction volume of 50 μl in 1× reaction buffer supplied by the manufacturer. Thermal cycling conditions included an initial template denaturation at 94 °C for 2 min, then 40 cycles of 94 °C for 30 s, 56 °C annealing for 30 s, 72 °C for 2 min; followed by a 72 °C extension for 15 min. PCR products were gel-purified in 1.5% NuSieve agarose gels (Cambrex, Walkersville, MD) and gel-slices of the amplicons were frozen at −80 °C and then extruded from the gel by centrifugation.

2.5. Sequencing and data preparation

Nucleotide sequencing was performed with BigDye Terminator Cycle Sequencing Kits on a 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). Sequence reaction volumes totaled 5 μl and included 1.5 μl DNA template, 0.25 μl sequencing primer (2.5 pmol), 0.5 μl BigDye Terminator, 1.0 μl BigDye sequencing buffer and 1.75 μl sterile distilled water and cycle sequenced according to the manufacturer’s instructions. PCR primers were used to sequence 5′-tef1, whereas an approximately 950 bp internal segment of Bloc was sequenced with primers B22U (5′-GTCGCG GCCAGGCAACT) and B22L (5′-AGATTCGACACGTCACATT). Sequence data was edited and assembled with Sequencer 4.1 (Gene Codes, Ann Arbor, MI) and aligned in the Megalign module of DNASTAR 5 (Lasergene, Madison, WI) and output in the Nexus file format.

2.6. Molecular phylogenetic identification

The phylogenetic diversity of Metarhizium and Beauveria strains recovered from the experimental field treatments was inferred by maximum parsimony (MP) and MP bootstrap analysis of 5′-tef1 and Bloc sequences, respectively. All MP searches for the shortest trees employed tree-bisection and reconnection branch swapping (TBR) and 1000 random sequence addition replicates. Nonparametric bootstrapping was conducted to assess clade support, and employed 1000 pseudo-replicates of the data, 10 random addition sequences per replicate and TBR branch swapping. An initial screen of the Metarhizium 5′-tef1 sequences was undertaken to assess sequence haplotype diversity (not shown) and a subset of representative isolates was selected for further analysis. A 47-taxon 5′-tef1 sequence matrix of 746 bp was created for Metarhizium that included 18 representative experimental strains and 29 authenticated

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Table 1

<table>
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<tr>
<th>Crop</th>
<th>Field Coordinates</th>
<th>County</th>
<th>Crop</th>
<th>Field Coordinates</th>
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<td></td>
<td>N44 26.809 W123 22.649</td>
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<td>Benton</td>
<td></td>
<td>N5 16.826 W122 44.892</td>
<td>Clackmas</td>
</tr>
</tbody>
</table>

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Metarhizium strains identified to species by Bischoff et al. (2009). Analysis of Beauveria Bloc sequences included all field-collected strains and the aligned Bloc data matrix was 985 bp. Phylogenetic species within B. bassiana have not been formally described, hence an ad hoc system is used to distinguish well-supported terminal clades that likely represent discrete phylogenetic species. Accordingly, exclusive terminal clades and branches are referred to using an alphanumeric coding system that includes a four letter abbreviation of the Latin binomial followed by a hyphen and Arabic numerals as clade identifiers in order of their discovery (e.g., Bbas-1, Bbas-2 ...). BLAST searches either to published Bloc sequences (Meyling et al., 2009) or unpublished data (Rehner, unpublished data) were used to determine the status of the different Beauveria phylogenetic terminals.

2.7. Larval bioassay

Four isolates each of Metarhizium guizhouense, Metarhizium robertsii, Metarhizium brunneum and three isolates of Metarhizium flavoviride var. pemphigi (only three isolates were isolated in this study) were randomly selected and screened against 8–10 weeks (5th instar) O. sulcatus using a modified version of the procedure outlined by Bruck (2004b). O. sulcatus larvae were obtained from a laboratory colony maintained at the USDA-ARS Horticultural Crops Research Laboratory, Corvallis, OR (Fisher and Bruck, 2004). The isolates used in the bioassay were derived from single-spore subcultures and were grown on PDA at 28 °C in complete darkness until sporulation. One plate of each isolate (15 × 100 mm) was flooded with 10 ml of a sterile 0.1% Tween 80 solution and the spores removed by gentle agitation with a sterile loop. Hemocytometer counts of all spore suspensions were made and their concentrations were adjusted to 5 × 10^5 spores/ml. The experiment contained four replicates of each treatment each containing five larvae and arranged in a randomized complete block design. Larvae were individually submerged into 1 ml of spore suspension (used only once) for 1 min and placed on filter paper to remove excess solution. Larvae were then placed individually into 29 ml plastic cups (Sweetheart Cup Co., Owings Mills, MD) with artificial diet (Fisher and Bruck, 2004) and incubated at 21 °C for 14 d, at which time larvae were observed to determine mortality. Mortality was defined as cadavers that showed evidence of sporulation by 2 weeks. The bioassay was performed twice on separate days using fresh spore suspensions for each test. All experiments included an untreated control (0.1% Tween 80). The arc-sine transformations of the percentage of larvae infected with each species of Metarhizium (i.e. sporulating cadavers) in the larval bioassays were analyzed using the General Linear Models Procedure (SAS Institute, 1999). An arc-sine transformation of the square root of the percentage larval infection was performed to stabilize the variances and a t-test was used to separate means (Snedecor and Cochran, 1989).

3. Results

3.1. Fungal entomopathogen survey

Four species of Metarhizium were isolated from the rhizosphere of plants collected in the Willamette Valley, OR: M. brunneum (Petch), M. robertsii (J.F. Bisch., Rehner & Humber), M. guizhouense (Q.T. Chen & H.L. Guo) and M. flavoviride var. pemphigi (W. Gams & Rozsypa) (Fig. 1). Strawberries and Christmas trees had the greatest number of root samples colonized and the greatest species richness of fungal species colonizing their rhizosphere (Fig. 2). A total of 39 of the 60 Christmas trees samples and 30 of the 70 strawberry samples were colonized. Christmas trees were colonized by all four species of fungi: M. brunneum (3), M. robertsii (11), M. guizhouense (24) and M. flavoviride var. pemphigi (1). Strawberries were also colonized by all four species: M. brunneum (26), M. robertsii (1), M. guizhouense (1) and M. flavoviride var. pemphigi (2) (Fig. 2). Blueberries (n = 109) and grapes (n = 100) had the lowest diversity and number of samples colonized of the four plant types. Only 12 of the blueberry samples and 13 of the grape samples were colonized. Blueberries were colonized by M. brunneum (10) and M. guizhouense (2). Grapes were colonized by M. brunneum (7), M. robertsii (4) and M. guizhouense (2) (Fig. 2).

Nine distinct Beauveria taxa were isolated including Beauveria brongniartii (n = 6) and Beauveria “Clade C” (n = 1) and seven phylogenetic species of B. bassiana: Bbas-16 (n = 31), Bbas-8...
(n = 3), Bbas-4 (n = 2), Bbas-2 (n = 4), Bbas-18 (n = 4), Bbas-indet 1 (n = 1), Bbas-indet 2 (n = 2), (Fig. 3). Three B. bassiana phylogenetic species are unique to this study including Bbas-16, Bbas-indet 1, Bbas-indet 2 (Fig. 3). Less than four rhizosphere samples were colonized by any Beauveria species except for Bbas-16, which colonized 29 of the 100 grape rhizosphere samples (Fig. 4).

3.2. Larval bioassays

All four species of Metarhizium bioassayed were pathogenic to O. sulcatus larvae and sporulated within 2 weeks of inoculation while none of the control larvae sporulated (Table 2). Excess mortality (dead larvae that did not sporulate) in the larval bioassay was never greater than 5.8%. Larval mortality was significantly greater in the larvae treated with M. brunneum and M. robertsii as compared to the control. However, M. flavoviride var. pemphigi and M. guizhouense did not cause significantly more larval sporulation when compared to the control (Table 2).

4. Discussion

This is the first study to specifically survey entomopathogenic fungi in the rhizosphere of crop plants under agricultural field conditions. Previous studies have sampled naturally-occurring entomopathogenic fungi in the soil (Bing and Lewis, 1993; Bidochka et al., 1998; Bruck, 2004b; Chandler et al., 1997; Harrison and Gardner, 1991; Klingen et al., 2002; Shapiro-Ilan et al., 2003; Meyling et al., 2009) but none have specifically targeted the rhizosphere. Our results demonstrate that multiple species of Metarhizium and Beauveria are frequently present in the rhizosphere and further suggest that certain species of Metarhizium and Beauveria may be significantly associated with the rhizospheres of particular plants as was observed in the case of strawberry, blueberry, grape and Christmas trees. Metarhizium and Beauveria are both common in the rhizosphere and the level of observed diversity of Beauveria spp. parallels that observed in soils as a whole (Meyling et al., 2009). Although we found that certain species of entomopathogenic fungi are associated with the rhizosphere, our study does not allow us to conclude whether these associations are a result of the plant type alone or are also influenced by other biotic and abiotic factors such as location in the field, availability of insect hosts, soil type, soil pH, soil moisture content and soil temperature (Jaronski, 2010). However, it seems plausible that matching of the prominent fungal species for use on a crop could enhance control of root-feeding insects.
Rhizosphere competence, the ability of an organism to show enhanced growth in response to developing roots (Schmidt, 1979), has been documented several times for Metarhizium that colonizes the rhizosphere of diverse plant species. In particular, we found that Metarhizium spp. predominate in agricultural habitats (Bidochka et al., 1998). In a separate study, Beauveria was isolated from the wheat rhizosphere in Australia (Sivasithamparam et al., 1987) and higher levels of inoculum were recovered from the first 6 cm of the soil profile containing clover roots than from the lower soil profile, suggesting Beauveria colonizes the clover rhizosphere (Brownbridge et al., 2006). Results of the present study demonstrate that Beauveria species occur frequently in the rhizosphere of diverse plant species. In particular, we found that Bb-as-16 was significantly associated with grape, indicating that this Beauveria clade is a common rhizosphere colonizer of grape. However, none of the other Beauveria taxa isolated in this study were significantly associated with any of the other plants sampled occurring at relatively low frequency. Interestingly, grapes were the only plant surveyed that were not significantly associated with at least one species of Metarhizium. Although it is not known whether entomopathogenic fungi interact competitively in the soil environment, this result suggests that Bb-as-16 may exclude Metarhizium in the grape rhizosphere but not on the other plants sampled in this study. Because grape vineyards are relatively undisturbed habitats as compared to blueberry, strawberry and Christmas tree fields, vineyards may have soil and rhizosphere microhabitats similar to those found in forest habitats that also experience little soil disturbance and favor the prevalence of B. bassiana (Bidochka et al., 1998). Further studies are planned to determine whether the Beauveria and grape association is due to the negative interaction of fungal entomopathogens or to a positive interaction between Beauveria and grapes.

The advantages associated with using fungal entomopathogens that are rhizosphere competent on the target crop plant could have significant economic impacts. Rhizosphere competent fungal entomopathogens incorporated into soil used during plant propagation would lead to a 10-fold reduction in the amount of fungal inoculum required to provide plant protection for container-grown nursery stock (Bruck, 2010). This reduction would drastically reduce the cost of using fungal entomopathogens to control root-feeding pests. The use of fungal entomopathogens that have a significant association with the target crop and are virulent against the target pest may lead to enhanced control due to

### Table 2

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Percent mortalityb</th>
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<tr>
<td>M. brunneum</td>
<td>19.4 ± 21.8bc</td>
</tr>
<tr>
<td>M. guizhouense</td>
<td>13.1 ± 19.4ac</td>
</tr>
<tr>
<td>M. robertsi</td>
<td>26.3 ± 23.5b</td>
</tr>
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<td>M. flavoviride var. pemphigi</td>
<td>6.7 ± 12.7a</td>
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<tr>
<td>Control</td>
<td>0 ± 0a</td>
</tr>
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* Bioassays performed at 5 × 10⁵ spores/ml.

b Means followed by the different letters are significantly different (P < 0.05; SAS Institute, 1999).

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**Fig. 4.** The percent of blueberry, Christmas trees, grape and strawberries samples collected from fields throughout the Willamette Valley, OR colonized by nine Beauveria taxa. Bars with different letters are significantly different (P < 0.05; SAS Institute, 1999) for each plant type.
increased persistence and growth. However, it is probably not economically feasible to develop commercial isolates of unique entomopathogenic fungi isolates for use on specific plant types. Therefore, in order to increase their efficacy, persistence and performance, selection of new biocontrol strains to control soil-dwelling insect pests should target fungal species demonstrated to be naturally associated with a wide range of different target plants. In addition to aiding in the selection of entomopathogenic species for commercial development, this study sheds additional light on the specific microhabitat preferences and ecology of fungal entomopathogens. While our understanding of the ecology and biological significance of fungal entomopathogens in the rhizosphere is only beginning to take form, it is clear that an increased understanding of this relationship is likely to be an important piece in the puzzle of microbial control of soil borne insects. The ramifications of this relationship are potentially significant. Thus, improved knowledge of entomopathogen’s ecology in niches within the rhizosphere could lead to improved deployment, formulation and efficacy against soil borne insect pests (Vega et al., 2009). Future research will focus on identifying entomopathogenic fungal isolates that have both ecological and pest hosts that can be developed for the control of root-feeding insects.

References


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