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Fungal entomopathogens in the rhizosphere

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Abstract The ecology of fungal entomopathogens in the rhizosphere is an understudied area of insect pathology. The rhizosphere is the region of soil in which the release of root exudates influences the soil microbiota, and may provide a favorable environment for fungal entomopathogens. The objective of this review is to bring together the relatively scant data available to date on the subject of fungal entomopathogens colonizing the rhizosphere and to highlight the importance of these findings. Gaining a better understanding of the ecology of fungal entomopathogens in the rhizosphere will help in the development of successful microbial control strategies against root-feeding insect pests.

Keywords *Metarhizium anisopliae* · *Beauveria bassiana* · Fungal ecology · Rhizosphere competent

Introduction

The rhizosphere encompasses a few millimeters of soil surrounding the plant root, an area where multifaceted ecological and biological processes take place. It is in the rhizosphere that complex interactions between roots, root exudates, beneficial and pathogenic microorganisms, and invertebrates take place. Hiltner (1904) was the first to define the "rhizosphere effect" by observing that the number and activity of microorganisms increased in the vicinity of plant roots. A large array of microbes can inhabit the rhizosphere and it is widely accepted that members from all microbial groups perform important functions in the rhizosphere (Giri et al. 2005). However, most studies of rhizosphere microbiology have focused on bacteria and fungi (Bowen and Rovira 1999). Two types of microbial interactions are recognized in the rhizosphere, those based on dead plant material (detritus-based) affecting nutrient and energy flows, and those based on living plant roots (Barea et al. 2005). Root exudates fall into two main classes of compounds: (1) low-molecular weight compounds such as amino acids, organic acids, sugars, phenolics, and other secondary metabolites, and (2) high-molecular weight compounds such as polysaccharides and proteins (Marschner 1995). Bais et al. (2006) published a comprehensive review on the role of root exudates on interactions between plant roots and other plants, microbes, and nematodes present in the rhizosphere.

There are three separate, but interacting, regions that make up the rhizosphere: the outer rhizosphere, the rhizoplane and the root (Kennedy 1998; Bowen and Rovira 1999). The outer rhizosphere contains the soil that is loosely adhered to the roots and is the

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region where the root exudates influence the soil microbiota. The rhizoplane is the portion of the rhizosphere directly in contact with the root surface resulting in the soil being tightly adhered to the roots. The roots themselves are also an important component of the rhizosphere, particularly for endophytic microorganisms (Kennedy 1998; Bowen and Rovira 1999). Because of the secluded nature of the rhizosphere it is an under-studied area of science. However, even in light of this fact, there have been significant discoveries particularly in the areas of the biological control of root pathogens (Whipps 1997, 2001) and phytoremediation (Pilon-Smits 2005).

Entomopathogenicity is a lifestyle that has arisen and been lost multiple times in many fungal lines (Roberts and Humber 1981; Rehner and Buckley 2005; Humber 2008). Hypocreales contains the largest number of fungal entomopathogens including two of the most widely studied, *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycete: Hypocreales) and *Metarhizium anisopliae* (Metchnikoff) Sorokin (Ascomycete: Hypocreales) both of which have been used for the microbial control of a wide array of foliar and soil-borne invertebrate pests (Lacey and Kaya 2007). Most studies have focused on the use of these fungi as replacements for chemical insecticides with little consideration of their ecological niche in the environment. The successful use of these fungal entomopathogens as microbial insecticides has been sporadic, due in large part to our incomplete understanding of their ecology. While commercial microbial control products based on *B. bassiana* and *M. anisopliae* have been registered around the world, they are used primarily in small niche markets and not large acreage crops. Several factors have limited the adoption of microbial control agents in the industrialized world including: regulatory constraints, activist resistance, benign and efficacious chemicals and limited research funding (Lord 2005). Other factors include inconsistent control, poor persistence, erratic product quality, poor shelf life and elevated costs relative to chemicals. To be effective, biological control agents must proliferate in the environment; a fundamental difference with chemical agents (Nelson et al. 1994). As a discipline, insect pathology must attain a better understanding of the ecology of fungal entomopathogens in order to improve the chances of success in agricultural production systems (Jaronski 2007; Vega et al. 2009). Entomopathogenic fungal isolates have traditionally been selected for development as microbial control agents based on laboratory bioassay results. Little emphasis has been placed on understanding the ecology of individual isolates. A preoccupation with killing insect pests has blinded us to the importance of fungal ecology when screening, selecting and releasing fungal entomopathogens in the field.

The soil has long been considered the natural reservoir for fungal many entomopathogens (Harrison and Gardner 1991; Bing and Lewis 1993; Chandler et al. 1997; Bidochka et al. 1998, 2001; Klingen et al. 2002; Shapiro-Ilan et al. 2003; Bruck 2004). Isolating fungal entomopathogens from soil offers insight into their biodiversity and provides a pool of potential microbial control agents. Traditionally, isolation is followed by bioassays against target pests in the laboratory to identify the isolate with the lowest LC_{50} and LT_{50} values. A much needed third step, following isolation and laboratory bioassays, should involve the characterization of the ecological constraints of the candidate isolates relative to the environment in which pests are being targeted. Understanding the dynamic interactions between the insect pests, the fungi and the host plant should be important considerations in the development and understanding of fungal entomopathogens as microbial control agents. History provides us with clear examples of the benefits of understanding fungal ecology for enhanced microbial control of insects. Lewis and colleagues (Bing and Lewis 1991, 1992) observed that *B. bassiana* grew endophytically within the green tissues of *Zea mays* L. (Cyperales: Poaceae). They also demonstrated that endophytic isolates of *B. bassiana* effectively controlled European corn borer, *Ostrinia nubilalis* (Lepidoptera: Crambidae; Lewis et al. 2002) while being non-pathogenic to *Z. mays* (Lewis et al. 2001). This pioneer research has in recent years led to investigation of *B. bassiana* as an endophyte of a wide variety of plants (Vega 2008). Insect pathology is not the only discipline to benefit from an enhanced understanding of microbial ecology. In the field of plant pathology, the "disease triangle" is a central concept based on the principle that disease is the result of an interaction between a host, a pathogen, and the environment (McNew 1960; Agrios 2005; Jones 1998). Insect pathologists developing microbial control programs would benefit greatly by integrating the disease triangle concept into their studies.

The objective of this review is to bring together the relatively scant data available to date on the ecology of fungal entomopathogens. Because this chapter is focused on fungal entompathogens in the rhizosphere, I will limit the discussion to the control of root-feeding insects.

The rhizosphere as a key microenvironment for fungal entomopathogens

Rhizosphere competent microorganisms are those that show enhanced growth in response to developing roots (Schmidt 1979). The discovery of *M. anisopliae* as rhizosphere competent was serendipitous (Hu and St. Leger 2002). Field trials by Hu and St. Leger (2002) were designed to determine the fate of fungal clones of *M. anisopliae* in the field. This was accomplished by employing a *gfp* gene driven by a constitutive promoter which strongly labeled the fungus with no impact on fungal growth or pathogenicity. Samples were collected from a variety of locations in and around the field to monitor for fungal distribution and persistence. Soil samples were collected 4–5 cm from, as well as adjacent to the cabbage taproot. During the six months following fungal application, the fungal titer in the bulk soil decreased from 10^5 propagules g^{-1} in the top 3 cm of soil to 10^3 propagules g^{-1}. However, fungal titers in the rhizosphere remained at 10^5 propagules g^{-1} six months after fungal application resulting in a 100:1 ratio in fungal densities between the rhizosphere and bulk soil (Hu and St. Leger 2002). The rhizosphere effect was most pronounced in the top 3 cm of soil and may be explained by a combination of two factors: (1) roots were most numerous in the top 3 cm of soil, and (2) fungal spores applied to the field were concentrated in the upper soil profile.

At the time that the Hu and St. Leger (2002) manuscript was published, we were performing experiments to determine the persistence of *M. anisopliae* (F52, Novozymes Biologicals Inc., Salem VA, USA) in bark and peat-based soilless potting media. Subsequent to learning that at least one isolate of *M. anisopliae* was rhizosphere competent, we sought to determine if *M. anisopliae* (F52) colonized the rhizosphere of *Picea abies* (L.) Karst. 'Nidiformis' (Pinales: Pinaceae). On each of the subsequent sample dates the difference between the fungal population in the rhizosphere and surrounding bulk media was significantly greater than zero, indicating that not only did *M. anisopliae* colonize the rhizosphere of *P. abies*, but the fungal population responded favorably to the rhizosphere microenvironment. The mean difference in *M. anisopliae* population levels between the rhizosphere and bulk soil ranged from 0.65 to 1.28 $\log_{10}$ CFU g^{-1} media. Data analysis of the mean difference between the rhizosphere and bulk media fungal population of each plant sampled showed that potting media type was the only parameter measured that had any significant effect on the size of the difference observed. The difference in *M. anisopliae* population levels between the rhizosphere and bulk media was greatest in the peat-based potting media on three of the five sample dates (Bruck 2005). Positive response to root exudates by *M. anisopliae* in the field was also suggested by Klingen et al. (2002), although the fungal population in the rhizosphere was not quantified. Studies of *M. anisopliae* population dynamics in the rhizosphere and surrounding bulk soil help describe the density as well as the temporal and spatial dynamics of the inoculum in soil. A more complete understanding of the relationship between the density of fungal entomopathogen inoculum and insect disease incidence is critical to understanding the outcome of microbial control efforts.

Subsequent studies demonstrated isolate variability in rhizosphere competence between plants. Studies were performed to determine the "rhizosphere host range" of F52 as well as *M. anisopliae* isolates collected from nursery soils in Oregon, USA (Bruck 2004). Bare root cuttings of *P. abies*, *Picea glauca* (Moench) Voss (Pinales: Pinaceae) and *Taxus baccata* L. (Taxales: Taxaceae) were planted into soilless potting media (Sunshine Mix #3, Sun Gro Horticulture, Bellevue, WA, USA) incorporated with one of three *M. anisopliae* isolates (F52, IP99, IP285). Four plants from each treatment were randomly selected at 6, 10 and 14 weeks after planting and the fungal population in the bulk and rhizosphere soil determined as described by Bruck (2005). The bulk soil populations of all isolates remained relatively steady or declined over the 14 week period (Figs. 1, 2, 3). The rhizosphere population response of each isolate to the various plant species was distinctive. The isolates F52 and IP99 were rhizosphere competent on the roots of *P. abies* with their populations increasing

nearly 10-fold over a 14 week period. However, the rhizosphere population of IP285 on the roots of *P. abies* remained flat (Fig. 1). These data confirm our earlier work demonstrating a significant population increase of F52 in the rhizosphere of *P. abies* (Bruck 2005). All of the isolates tested colonized the rhizosphere of *P. glauca* with a nearly 10-fold increase in their populations over the 14 week period (Fig. 2). None of the isolates tested responded favorably to the rhizosphere of *T. baccata* over the course of 14 weeks (Fig. 3).

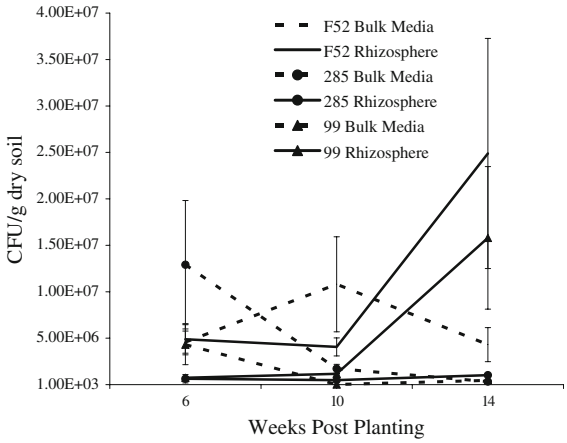

Fig. 1 Fungal population ± SE (cfu g^{-1} dry soil) of three *M. anisopliae* isolates in the bulk soil and the rhizosphere soil of *Picea abies* 6, 10 and 14 weeks after planting in fungal inoculated soil

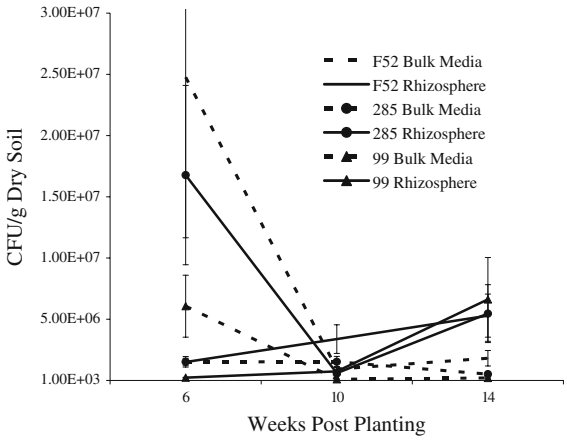

Fig. 2 Fungal population ± SE (cfu g^{-1} dry soil) of three *M. anisopliae* isolates in the bulk soil and the rhizosphere soil of *Picea glauca* 6, 10 and 14 weeks after planting in fungal inoculated soil

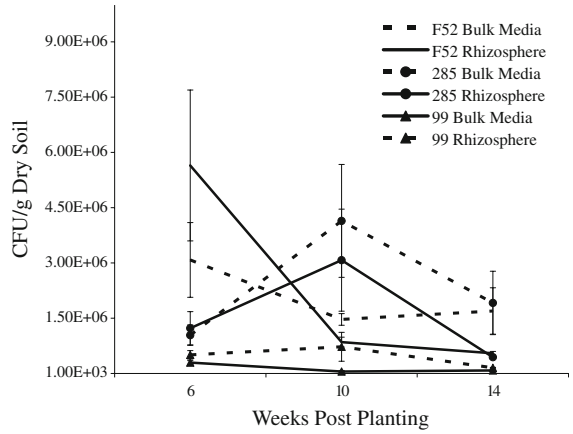

Fig. 3 Fungal population ± SE (cfu g^{-1} dry soil) of three *M. anisopliae* isolates in the bulk soil and the rhizosphere soil of *Taxus baccata* 6, 10 and 14 weeks after planting in fungal inoculated soil

Tritrophic interactions

Tritrophic interactions are well described for terrestrial systems (Sabelis and van de Baan 1983; Dicke et al. 1990; Turlings et al. 1990; Dicke et al. 1993; Turlings et al. 1995; Kessler and Baldwin 2001). In above-ground systems, herbivore feeding elicits systemic production of secondary metabolites by plants that serve as attractants to predators and parasitoids (Turlings and Tumlinson 1992; Dicke et al. 1993). Tritrophic interactions may also involve entomopathogens, plants, and insects (Cory and Ericsson 2009). Currently, it is unclear if plants manipulate 'bodyguard' entomopathogens similarly to their manipulation of predators and parasitoids (Sabelis et al. 1999; Elliot et al. 2000). While bodyguard traits are yet to be demonstrated with microbial entomopathogens, these microorganisms are clearly involved in tritrophic interactions and that multitrophic relationships also exist (Cory and Hoover 2006). One example of the complex interactions occurs between secondary plant metabolites and the fungal entomopathogen *Neozygites tanajoae* Delalibera Jr., Humber & Hajek (Zygomycetes: Entomophthorales) used in the control of cassava green mites *Mononychellus tanajoa* (Bondar) (Acari: Tetranychidae). Cassava green plant volatiles suppress the germination of *N. tanajoae* in the absence of mite feeding (Hountondji et al. 2005). However, plant volatiles released in response to green mite feeding on leaves trigger

conidiation, allowing the fungus to release infective spores when mites are present (Hountondji et al. 2005).

Tritrophic interactions have been found to operate below ground as well. One case involves the entomopathogenic nematode, *Heterorhabditis megidis* Poinar Jackson & Klein (Rhabditidae: Heterorhabditidae) and its orientation to black vine weevil *Otiorhynchus sulcatus* (F.) (Coleoptera: Curculionidae) larvae. Boff et al. (2001) observed *H. megidis* attraction towards strawberry plants fed upon by black vine weevil larvae. However, they were unable to determine if the orientation was due to chemical cues emitted from the plant. The attraction of *H. megidis* to chemical cues released by the conifer *Thuja occidentalis* L. (Pinales: Cupressaceae) fed upon by black vine weevil larvae feeding was confirmed by van Tol et al. (2001). Since these initial findings, the production of natural enemy attractants in response to root herbivory has been identified in turnips (Neveu et al. 2002), tulips (Aratchige et al. 2004) and corn (Rasmann et al. 2005).

There is contradictory evidence in the literature concerning the ability of fungal entomopathogens in the soil to influence insect behavior. Villani et al. (1994) observed that Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae) oviposited preferentially on bare soil treated with *M. anisopliae* mycelia over non-inoculated bare soil, possibly in response to CO_2 released during mycelial growth. However, Japanese beetle grubs avoided regions of sod treated with *M. anisopliae* (Villani et al. 1994). Rath (2000) found that isolates of *M. anisopliae* vary in their repellency in the laboratory and field against termites. The termites *Reticulitermes flavipes* (Kollar) and *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae) were attracted to *M. anisopliae* mycelial preparations and volatile extracts (Engler and Gold 2004). Mole crickets (Orthoptera: Gryllotalpidae) modified their behavior in response to *M. anisopliae* and *B. bassiana* incorporated into soil so as to reduce their exposure to these fungal entomopathogens (Villani et al. 2002; Thompson and Brandenburg 2005). Rath (2000) as well as Thompson and Brandenburg (2005) demonstrated that termite and cricket avoidance behavior, respectively, was dependent on the fungal isolate, which may partially account for the behavioral differences observed among fungal isolates and insect species. More recently, wireworms *Agriotes obscurus* L. (Coleoptera: Elateridae) were repelled by *M. anisopliae*-contaminated soil at a rate that increased with conidia concentration in the soil. However, the rate of emigration was reduced when food was present (Kabaluk and Ericsson 2007a).

St. Leger (2008) speculates that *M. anisopliae* could provide a "repellent barrier" around plant roots which would provide more effective protection to the plant than direct fungal infection of the herbivore, primarily due to the time lag between infection and cessation of feeding. This may well be the case with some fungal entomopathogen isolates, however. The opposite phenomenon in which insects are attracted to plants when their rhizosphere is colonized may also occur (Kepler and Bruck 2006). When placed in a two-choice soil olfactometer, black vine weevil larvae were significantly more attracted to *P. abies* roots growing in *M. anisopliae* inoculated potting media than plants grown in uninoculated media, revealing a tritrophic interaction that differs significantly from previous reports (Kepler and Bruck 2006). In our studies, it was not a natural enemy whose behavior was altered in response to secondary plant metabolites (Turlings and Tumlinson 1992; Dicke et al. 1993; Boff et al. 2001; van Tol et al. 2001; Rasmann et al. 2005), but rather the behavior of the pest itself. From an evolutionary standpoint of the fungal entomopathogen this makes sense as *M. anisopliae* spores in the soil are not able to actively seek out insect hosts. If *M. anisopliae* is in fact utilizing the rhizosphere as a bridge between insect hosts, preferentially attracting hosts to the fungus in the rhizosphere may substantially shorten the length of the bridge. Unfortunately, we can only speculate on whether the fungus or plant is the source of the attractive compound(s). The evidence seems to indicate that the plant in association with the fungus produces compounds attractive to black vine weevil larvae. However, it is also plausible that when colonizing the rhizosphere, the fungus produces attractive compounds that are not produced in the absence of plant roots. There may be an evolutionary benefit to the plant in having root-feeding insects attracted to fungal colonized plants in a community in which there is not 100% fungal colonization. In such a scenario, root-feeding insects preferentially feed on roots colonized with fungal entomopathogens

subsequently becoming infected which results in a net reduction in root-feeding in the plant community.

A bodyguard interaction between host plant and the herbivore via an entomopathogen is by definition an indirect one (Elliot et al. 2000). Plants may have an indirect impact on entomopathogens by: (1) maintaining a population of the entomopathogen, (2) increasing contact rate between the insect and the entomopathogen and, (3) by increasing the susceptibility of the insect to the entomopathogen (Elliot et al. 2000). In the case of *M. anisopliae*, fungal propagules in the rhizosphere increase in response to root exudates (Hu and St. Leger 2002; Bruck 2005) and the presence of the fungus in the rhizosphere, at least in some cases, results in increased exposure of insects to the fungus (Kepler and Bruck 2006). An increase in insect susceptibility to fungal entomopathogens in the rhizosphere has yet to be demonstrated, but all three of the above scenarios outlined by Elliot et al. (2000) need not occur for the bodyguard interaction to be successful. In addition, the employment of a fungal entomopathogen as a bodyguard by a plant must result in a net positive return on investment, must complement the plants other defenses, and the investment must be secure (Elliot et al. 2000). Preliminary data suggest that at least in the case of *M. anisopliae* colonizing the rhizosphere of *P. abies*, there is no measurable cost to plant fitness (Kepler and Bruck, unpublished data). Cooperation between host plants and microorganisms should benefit both partners, given their differing resource needs and metabolic capabilities (Hoeksema and Schwartz 2003). However, these mutual benefits do not guarantee that the cooperation is evolutionarily stable (Kiers and Denison 2008).

Role of fungal entomopathogens in the rhizosphere for controlling root-feeding insects

We have demonstrated the pest management potential of rhizosphere-competent fungal entomopathogens (Bruck 2005). Colonization of the rhizosphere of *P. abies* by a rhizosphere competent isolate of *M. anisopliae* provided nearly 80% control of black vine weevil larvae within two weeks of exposure to inoculated roots (Bruck 2005). This work was the first to demonstrate that roots colonized with a fungal entomopathogen resulted in high levels of insect infection through root feeding. Hu and St. Leger (2002) also noted that the carrying capacity of *M. anisopliae* (2575-GFP) in the cabbage rhizosphere (10^5 propagules g^{-1}) was higher than the LC_{50} value of the isolate against a number of insect pests. While our understanding of the ecology and significance of *M. anisopliae* in the rhizosphere is in its infancy, it is clear that an increased understanding of this relationship is likely to be an important aspect in the microbial control of root-feeding insects. Currently, data on the pest management potential of rhizosphere competent fungal entomopathogens are scant. However, the prospective ramifications of this relationship are tremendous. A simple calculation of the economic benefits that can be realized by utilizing rhizosphere competent fungal entomopathogens yields savings significant enough to warrant further investigation. For example, a grower of container-grown ornamentals utilizes approximately 10× the amount of potting media annually to grow production plants as is used in the propagation of new plant material at their operation. The use of a rhizosphere competent fungal entomopathogen incorporated into soil during plant propagation would result in a 10-fold reduction in the amount of fungal inoculum required. The use of rhizosphere competent fungal entomopathogens could result in effective control of root-feeding insect pests without the added cost of treating the surrounding bulk soil with large numbers of fungal propagules. Great numbers of fungal entomopathogen propagules are applied or incorporated into soil for the control of root-feeding insects, most of which are not involved in control.

Soil adapting traits

Habitat and proximity to potential insect hosts are important driving forces in the population structure of *M. anisopliae* and *B. bassiana* (Bidochka et al. 1998, 2001, 2002; Humber 2008). Bidochka et al. (1998) found *M. anisopliae* occurred more frequently in agricultural habitats while *B. bassiana* was predominately isolated from forested habitats. Genomic analysis of *M. anisopliae* revealed two non-recombining lineages of *M. anisopliae* var. *anisopliae* in southern Ontario, Canada; one lineage typically occurred in agricultural soils while the other was more common in forest soils (Bidochka et al. 2001).

Recent analyses have determined that the two mutually exclusive groups reported by Bidochka et al. (2001) are *M. robertsii* and *M. brunneum* (Bischoff et al. 2009). Conversely Inglis et al. (2008), observed that two closely-related cosmopolitan genotypes of *M. anisopliae* var. *anisopliae* predominated urban, agricultural, and forest soils in southwestern British Columbia, Canada. The discrepancy between these studies may be the result of the geographic isolation which restricted emigration of *M. anisopliae* into southwest British Columbia (Inglis et al. 2008) or cryptic species (Bischoff et al. 2009). Within any particular habitat, it is not unreasonable to assume that rhizosphere colonization may play a key role in which fungal entomopathogens are present. Plants growing in soil containing fungal entomopathogens would result in long-term exposure of fungi to certain plant communities putting a tremendous amount of selection pressure on the fungi to select for those that can "bridge" the gap between insect hosts by persisting in the rhizosphere of plants in that particular habitat. As stated by Humber (2008) "Natural selection may also lead a fungus to an increasing or decreasing level of nutritional and biological adjustment to its food source; such adjustments could move a fungus in any direction along the nutritional continuum from beneficial to commensal to saprobic to parasitic to pathogenic associations with the source of its nutrients". Two differing sets of selection pressure appear to be at play on fungal entomopathogens: survival in soil and virulence towards insects (Prior 1992). A review by St Leger (2008) outlines the adaptations of *M. anisopliae* to life in the soil. *M. anisopliae* expresses a different subset of genes to persist and colonize insect and plant tissues suggesting that the ability to adapt to life in the soil and as an insect pathogen requires different subsets of genes (Wang et al. 2005). *M. anisopliae* produces two different proteins (MAD1 and MAD2) used for adhesion to insect and plant surfaces. MAD1 and MAD2 are differentially produced in response to insect hemolymph and plant root exudates, respectively. Expression of MAD1 and MAD2 in yeast cells allowed them to adhere to insect cuticle and a plant surface, respectively. *M. anisopliae* is able to adapt its adhesive properties to insects or plant roots through regulation, localization, and specificity control in the functional distinction between MAD1 and MAD2 (Wang and St. Leger 2007).

Conclusions

Jaronski (2007) considered the ecology of fungal entomopathogens in soil and stated "If a generalization can be made, it is that one simply cannot generalize." The result of any one study of the ecology of fungal entomopathogens in soil cannot be used to make broad generalizations on their ecological role. The soil habitat and all of the complex biotic and abiotic interactions that occur in the soil are extremely complex and it is evident that not all fungal entomopathogens are performing the same role. Our current knowledge serves as the foundation for future research to advance our understanding of the ecological niche of soil-borne fungal entomopathogens. Studies of fungal ecology in the rhizosphere to date have focused on *M. anisopliae*. However, natural rhizosphere colonization by *M. anisopliae* and *B. bassiana* readily occurs on a variety of plants (Bruck unpublished data). It is plausible that as research continues, other fungal entomopathogens will be isolated from the rhizosphere as well. Natural rhizosphere colonization indicates that this phenomenon is not an artifact of the relatively short duration or the inundative release of fungal spores into the environment that took place in studies to date. The employment of molecular approaches will provide better insight into the genotypic diversity and aid in our understanding of the ecology of naturally-occurring fungal entomopathogens in soil and the rhizosphere. Bischoff et al. (2009) recognized nine distinct phylogenic species with the *M. anisopliae* lineage. The ability to objectively differentiate cryptic species using molecular tools allows for systematic efforts to differentiate physiological and ecological features that may further differentiate these phylogenic species (Bischoff et al. 2009).

Much is left to be done to fully understand the role that rhizosphere competent fungal entomopathogens play in regulating pest populations and how we can use that knowledge to design and implement more effective microbial control programs. Questions of particular importance to consider are highlighted by Vega et al. (2009) and include the following: (1) Do plants benefit from a rhizosphere association with fungal entomopathogens? (2) Is the 'bodyguard' concept relevant in soil? If so, what is the signaling mechanism between trophic levels? (3) Do different phylogenetic groups of fungal entomopathogens

display different strategies in their association with plants? (4) How do soil-borne fungal entomopathgens interact between above and below ground ecosystems? (5) What is the mechanism of yield increases in *Z. mays* reported by Kabaluk and Ericsson (2007b)? *M. anisopliae* increased the stand density and fresh weight of *Z. mays* when conidia were applied to seeds prior to planting. Unfortunately, the mechanism for the yield increase is unknown. (6) Does plant diversity impact fungal entompathogen diversity at the landscape or local level, and what is its impact on natural pest control? In addition to the basic scientific questions posed above, there are a number of applied questions that require further investigation as well: (1) What is the most effective approach for inoculating roots with rhizosphere competent isolates? Approaches will need to be identified for plants propagated via seed, cuttings and tissue culture. (2) How long do rhizosphere competent isolates persist on the root system of annual and perennial plants? (3) Will the use of rhizosphere competent isolates provide consistent and acceptable levels of pest control?

Future prospects

Clearly, further investigation is necessary before we have even an elementary understanding of the ecology of fungal entomopathogens in soil. Early indications are that the rhizosphere, up until recently, has been an under appreciated niche for soil-borne fungal entomopathogens. A more complete understanding of fungal ecology is likely to aid in not only the development of the next generation of microbial control programs but may also lead to other benefits including increased yields (Kabaluk and Ericsson 2007b), direct disease antagonism, compatibility with other beneficial microorganisms in the rhizosphere (Jaronski et al. 2006), and plant growth promotion.

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