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Challenges in Using *Metarhizium anisopliae* for Biocontrol of Sugarbeet Root Maggot, *Tetanops myopaeformis*

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Abstract

Metarhizium anisopliae is under development as a microbial pest control agent of the Ulidid fly, *Tetanops myopaeformis* (Sugarbeet Root Maggot), the most serious sugar beet pest in the United States. The fungus can be deployed by several means to create a "minefield" of infectious spores in the habitat of young larvae migrating to the developing root: (1) placing conidia on/in the seed coat to allow the fungus to colonize the rhizosphere; (2) applying *Metarhizium* granules around the seed at planting, much like insecticide granules; or (3) applying an aqueous spray of spores at or before peak fly oviposition to a narrow band of soil at the base of plants, allowing spores to soak into the top cm of soil, where eggs are laid. A number of constraints could affect successful control by limiting *Metarhizium* survival before spores can contact larvae and influence conidial acquisition. We have examined several factors affecting *Metarhizium* performance: conidial and fungal granule concentrations in the soil; soil type (texture), moisture, and temperature influences on efficacy and persistence; the extent of rhizoplane/rhizosphere colonization; the effect of common planting-time fungicides; and interactions with a sample of rhizosphere-associated bacteria. The value of planting-time granule and preovipositional conidial sprays in high and low insect pressure situations was also determined in replicated field trials.

Introduction

The sugar beet, *Beta vulgaris* L., was introduced into the United States in the 1890s, and now encompasses some 570,000 ha across 12 states, with a total annual production of 30,624,000 tons, about half of all refined sugar produced domestically. Depending upon the locale, sugar beet is typically grown in a 2-year or 4 to 5-year rotation with cereals, legumes, alfalfa, potato and maize. The majority of sugarbeet farms are <200 ha, with about 40% <60 ha.

Perhaps the most significant insect pest of sugar beets is the sugarbeet root maggot (SBRM), *Tetanops myopaeformis* (Diptera: Ulididae), which is native to North America. Its original hosts were probably wild Chenopodiaceae, but as sugar beets became prevalent in the early 1900s the flies adapted. It was not until the 1950s that the fly was recognized as a pest. In the early 1970s it became a serious pest, esp. in the Red River Valley, between North Dakota and Minnesota. Today it seriously impacts approximately 278,500 ha (49% of total area). Untreated, it can cause up to 40% yield loss. The insect overwinters as a diapausing third-instar larva, 30-45 cm deep in the soil. In spring, as soils warm, the larvae break diapause, migrate to within 10 cm of the soil surface and pupate. Adults emerge shortly thereafter and migrate from the previous year's fields to the present year's sugar beets. Adult emergence and migration can be predicted with a degree-day model and monitored by use of orange sticky traps. The female lays her eggs in the top cm of soil immediately around the emergent sugarbeet seedling, usually about 5-6 weeks after planting. The main control tools historically have been chemicals; currently terbufos and chlorpyrifos are the principal insecticides, used at planting or to a lesser extent as a post-emergent lay-by treatment.

Aldicarb and phorate are used to a much lesser extent; esfenvalerate and zeta-cypermethrin sprays are sometime used to suppress adult flies.

Microbial control of SBRM has been the focus of U.S. Department of Agriculture efforts since the mid-1990s. Since 1994 research has focused on a series of *Metarhizium anisopliae* var. *anisopliae* isolates; the latest being tested is the recently commercialized F52 (Novozymes Biologicals, Salem VA). The F52 isolate is better known in Europe as BIPESCO 5, (G. Zimmerman, personal communication).

There are several ways to deploy a fungal control agent against sugarbeet root maggot. The key with all tactics is to create an infectious "minefield" of fungal spores to intercept newly hatched maggots as they migrate to the sugarbeet root. The standard approach has been to apply granules containing *Metarhizium* conidia at planting time, much in the same manner as chemical insecticide granules. The fungal conidia might be applied as a seed coat from which the fungus could colonize the growing root system. Or, one might wait until the flies move into the sugarbeet field to oviposit, then apply an aqueous spray of fungal conidia to the bases of the beet seedlings in a band-over-row spray, using conventional equipment.

There are some overarching practical constraints in deploying *Metarhizium*, or any microbial, against SBRM, to achieve market acceptance. First, a microbial needs good commercial characteristics (commercially feasible production, practical formulation(s), and satisfactory shelf life). Second, any microbial product needs also to conform to typical agricultural practices, equipment, amounts of formulation and carrier/acre applied to the crop. Major changes in farm practice, esp. ones that would require capital outlay to employ a microbial product, will be met with user resistance. A microbial product must also be cost competitive unless it offers some significant advantages; in sugar beets an upper cost limit may be on the order of US$50 per hectare. Many U.S. farmers operate in a chemical paradigm, leading to expectations of high-degree, rapid efficacy that are possible only with chemical insecticides.

Specific Challenges in the Use of *Metarhizium*

Metarhizium in a seed-coat application. Rhizosphere, and especially rhizoplane, colonization and subsequent sporulation by the fungus are prerequisite to the feasibility of this approach. The ubiquitous seed-coat fungicides cannot interfere with colonization and sporulation, nor can the many rhizosphere-/rhizoplane-inhabiting microorganisms. Once the previous challenges have been met, the conidia must be able to survive seed pelletization, very commonplace in this industry, although hand-mixed, ad hoc, seed coating used with *Rhizobium* might be feasible.

Using green fluorescent protein transformants, we have observed that *Metarhizium* and *Beauveria* can colonize the rhizoplane of young sugarbeet seedlings in vitro, in an agar-based system. However, in gnotobiotic media -- sterile clay soil, sterile potting mix, vermiculite + 10% Hoagland's Solution – rhizoplane colonization was not observed, regardless of whether conidia were applied to the seed coat or added to the medium. Root colonization was also not observed with chard (*Beta vulgaris var. cicla*), bean (*Phaseolus vulgaris*), or maize (*Zea mays*) seedlings. We determined that conidial germination was almost nonexistent in root exudate of 2-leaf sugar beets, but reached about 50% after 24 hr in exudate from 4-leaf beets, cabbage and chard. In contrast, germination was >95% in oat, rye or bean root exudate, in 1% neopeptone, or in Sabouraud dextrose broth. It is clear that a seed-coat approach may not be practical. We also examined the in vitro interactions between 30 rhizosphere bacteria and each of three isolates of *B. bassiana* and *M. anisopliae*. There were qualitative differences among the fungal species and isolates in their response to the various bacteria. A general trend appears to be greater inhibition of germination by Gram negative (G-) than Gram positive

(G+) species. Hyphal growth of the fungi was generally not inhibited by any of the bacteria. More G+ bacteria were inhibited by *Metarhizium* than by *Beauveria*, and fewer G- bacteria were inhibited by either fungus. The nature of the medium strongly affected the results.

Metarhizium as a planting-time granule. Granules must be compatible with farm practice, i.e., be of a size and bulk density that can be properly applied by existing equipment. Agricultural requirements mandate a minimal granule size of 0.5-1.5 mm diameter, and an upper practical limit of 22 kg granules/ha in sugar beets. (Conventional insecticide granules are applied at 11-17 Kg/ha.) Thus, the GranMet® -style fermentation substrate granules (AgriFutur s.r.l, Italy), based on barley, are not practical because they are larger than a pelleted sugarbeet seed. Pulverized spent substrate, on which the fungus was grown for conidial production, is more practical, but we have observed that the pulverization and mechanical size grading processes render the granules more susceptible to colonization by soil fungi once the granules have been applied, and it is difficult to create granules of a satisfactory size range without considerable wastage.

A critical concentration of granules is needed for high efficacy. In replicated bioassays using third-instar larvae in a clay soil typical of sugarbeet culture and at optimal moisture and temperature for the fungus, 4 or more granules (corn grit granules 0.5-1 mm diameter, coated with *Metarhizium* conidia and having a bulk density of 1400 granules/gram) per gram were needed for >90% efficacy (Jaronski, unpublished data). At 4 granules/cc soil one would need 158 Kg granules/ha if applied in a 12-15 cm band over the row and incorporated to a depth of 2.5 cm – clearly impractical and uneconomic. But the critical concentration of granules can be achieved at 11 Kg/ha when granules are applied using in-furrow approaches.

Permissive temperatures and soil moisture are required for *Metarhizium* outgrowth and sporulation; the latter process is crucial with mycelial granules. Fortunately, our studies have revealed that if soils are permissive for sugarbeet seed germination, conditions are permissive for the fungus.

Mefenoxam, hymexazole, and thiram are universally used in sugarbeet seed coats to protect the young seedlings from damping off pathogens. The latter is quite toxic to *Metarhizium* in the traditional *in vitro*, agar-based protocols. However, when *Metarhizium* granules are placed even < 5mm from a seed, with the full complement of fungicides, fungal outgrowth and sporulation are not inhibited on water agar or moist soil (Jaronski, unpublished data). The lag time of 6-8 weeks between planting (and *Metarhizium* granule application) and SBRM egg hatch requires good persistence of the *Metarhizium* conidia. This is achievable, particularly by a new form of mycelial granule developed by USDA (Jackson and Jaronski, unpublished).

Metarhizium in a post-emergence spray. The tactic here is to spray a conidial suspension into the soil around the base of the plants where the flies oviposit at the immediate onset of oviposition. Timing can be close to oviposition and egg hatch, as determined by degree day models and established surveillance methods, but there are disadvantages: (1) the conidia are restricted by carrier volume limitations to the top 5-10 mm of the soil surface, a zone exposed to extreme temperature and moisture fluctuations; and (2) the presence of neonate larvae in this zone is transient, limiting exposure to conidia. The critical concentration for high larval mortality is on the order of $1x10^6$ conidia/cc of soil, a level theoretically achievable with commercially acceptable rates of fungus. However, a complicating factor is the effect of soil texture and moisture on the infectivity of conidia, most likely mediated through pore structure. In one clay soil we observed a direct relationship between moisture (10, 15, 30% water holding capacity) and conidial infectivity; in other soils this relationship breaks down, lacking consistent correlation with the sand:silt:clay ratio. In contrast, the same relationship between moisture and infectivity holds in the case of a second clay, and in a loam, while in a clay loam, and in a sandy clay loam, increasing water content does not affect fungal infectivity.

However, infectivity is adversely affected in a sandy loam. In all cases conidial viability is unaffected. This complex relationship between soil texture, moisture, and conidial infectivity may present serious challenges to the broad application of this particular tactic in controlling SBRM. Fortunately, soil temperatures are permissive for infection and pathogenesis during the critical window of larval contact with conidia, and several years of field observations indicate that conidial persistence in the soil is sufficient to potentially affect most of the neonate larvae.

Several years of field trials evaluating at-planting applications of granules and ovipositional period sprays of conidia have shown us that *Metarhizium* can reduce root damage to the same extent as terbufos in *low* insect pressure situations. These are the situations where chemical insecticides are perhaps not really necessary but are still being used because of grower sentiment. Against high insect pressure, however, such as occurs very frequently in northeastern North Dakota and in central Idaho, efficacy is clearly insufficient. Integration of *Metarhizium* with other IPM tools is therefore needed; the fungus cannot be successfully used by itself. Coupling the fungus with resistant sugarbeet hybrids has not yet proved successful. In one trial, yield improvement from use of *Metarhizium* with an SBRM-resistant variety was not significantly different from *Metarhizium* with a susceptible cultivar (Jaronski and Campbell, 2006). Early planting, or use of a harrow or rotary hoe during cultivation, can help reduce SBRM impact, complementing use of *Metarhizium*; this approach still needs evaluation.

The most successful approach so far has been the use of the fungus with an oat or rye cover crop (Majumdar et al., 2003, 2004, 2006). Beet farmers in the southern Red River Valley have used such cover crops to control spring wind erosion. The cereal crop is planted just before the beets, and comes up before them. The cereal – rye or oats may be the best – is killed off with herbicides when it is 10-15 cm tall, during the normal course of the crop season. The cover crop does not seem to have any impact on yields when used in this way.

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