

***Metarhizium anisopliae* for biocontrol of sugarbeet root maggot: constraints and challenges.**
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ABSTRACT

The entomopathogenic fungus *Metarhizium anisopliae* has met with mixed success controlling the Sugarbeet Root Maggot, *Tetanops myopaeformis* (SBRM) in the field. A number of constraints, abiotic and biotic, could affect the successful outcome by affecting *Metarhizium* survival before larvae contact spores, and spore attachment/penetration into the insect. We examined several factors as they affect *Metarhizium* granule and conidial spray performance: soil type (texture), soil moisture, temperature, and pathogen concentrations in soil. Performance against high and low insect pressure was also determined in replicated field trials.

Metarhizium anisopliae strain MA1200 (ATCC 62176) has been the historic lead candidate 1998-2004; Strain F52 (Earth Biosciences) was identified as a better candidate in 2004. F52 is already registered in the U.S., for other insects, while MA1200 is not. The active ingredient of *Metarhizium* is the conidiospore. A larva must come into contact with a sufficient number of conidia to become infected – that is the bottom line for efficacy. The conidia germinate on and penetrate the cuticle within 24 hr., then the fungus proliferates inside the larva and kills it.

Metarhizium can be deployed in several different ways to achieve a critical objective: a “mine-field” of infectious spores in the habitat of young larvae, intercepting them as they move from egg to the developing root. One can (1) put spores on/in the seed coat; (2) apply *Metarhizium* granules around the seed at planting, much like insecticide granules; or (3) apply an aqueous spray of spores, at or before peak fly movement into the field, in a tight band-over-row to base of plants, soaking spores into the top 1-2 cm of soil, where the flies lay their eggs.

Seed-coat application challenges: Rhizosphere colonization by *Metarhizium* & subsequent sporulation are necessary, but as yet unproven, events; surviving seed pelletization is also a problem; seed-coat fungicides, esp. thiram, are a presumed problem. Determination of colonization is being pursued in our lab by use of gfp-transformed *Metarhizium* but work is yet at an early stage.

At-planting granular application challenges: Conidia stay put on granules. Therefore, It is a numbers game — numbers of granules per cc soil; seed fungicides are a possible problem; suitable moisture and temperature are needed for fungal regrowth on granules; and fungal persistence is needed until oviposition and egg hatch (4-6 weeks).

With granules, it's a numbers game. To examine the concentration:efficacy relationship we mixed various numbers of MA1200 granules with Savage silt clay wetted to -0.1MPa; one week later third instar SBRM larvae were added. Mortality was determined after another three weeks at 20-23° C. At least 4 grains/cc were necessary for > 90% efficacy. This level translates to 11 kg granules/ha when applied “Modified In-Furrow” (MIF) or 157 kg/ha in a 13 cm (5 in.) band-over-row application tilled 2-3 cm deep. The latter rate is not economically or practically feasible. While it is possible that the target 1st and 2nd instar larvae are more susceptible than 3rd instars, and lower rates are thus feasible, only in furrow applications of granules are economic.

Is there enough soil moisture for *Metarhizium* outgrowth? MA1200 granules were placed on 5 different types of sugarbeet field soils, wetted to 7.5-25% Water Holding Capacity (WHC) for each soil. Water Activity (Aw) was measured with Decagon® Aqualab moisture analyzer. After

one week at 25° C. granules were examined for *Metarhizium* “exflorescence” (outgrowth) and sporulation. In all soils, *Metarhizium* failed to grow out at moistures below Aw of 0.99 (-1.5 MPa). Only minor differences existed in the critical Aw among soils. In general, based on in situ measurements during 2001-2004 field trials, soils are moist enough in seed zone for *Metarhizium* exflorescence.

Is soil too cool/too warm for *Metarhizium* granules? Temperature tolerances of the two *Metarhizium* candidates, expressed as percent maximum radial growth (mm/day) on agar, were determined. “Good” growth occurs between ~20 and ~32° C. Below 20° C. growth slows, ceasing at ~8-10° C. Above 32° C. growth rapidly decreases. Observed soil temperatures 3 cm deep at Sidney MT and St. Thomas ND indicate that soil temperatures post planting result in slow, below optimal growth about half of each day; upper temperature limits of *Metarhizium* are rarely reached.

Are sugarbeet seed-coat fungicides a problem? To ascertain fungicide effect, MA1200 granules were placed at 0.5 cm intervals from either treated seed or filter paper disks impregnated with fungicide at the concentration found on a beet seed. After 1 week, granules were examined for characteristic *Metarhizium* outgrowth and sporulation. The test was repeated on a series of soils wetted to -0.1 MPa. In all cases *Metarhizium* on granules 0.5 cm from seed or disk grew and sporulated abundantly.

3. “Peak-fly” spray application challenges: Timing is closer to oviposition so good fungal persistence less important than with at-planting applications, but there is no amplification of conidial numbers as on granules. Placement is better than at-planting granules — spores are concentrated where oviposition and hatch occur, but spores don’t move. It’s a numbers game: spores/cc soil vs. \$/ha; one needs sufficient carrier to “water spores in”, > 20 gpa. Temperature, soil moisture and type can affect efficacy.

Can *Metarhizium* conidia persist long enough? The top 2 cm of soil in the sprayed band was sampled at 7-day intervals post-application and numbers of colony-forming units were determined by serial dilution plating onto selective dodine oatmeal agar (4 replicate plates of two independent dilution series). Soil temperatures at the 1-1.5 cm depth were measured with thermocouples attached to dataloggers. Soil moistures were measured from fresh daily samples in a Decagon® Aqualab moisture analyzer. *Metarhizium* titers decreased by less than 80% at one site, remained generally steady at a second and briefly increased to a steady state at the third site. These trends occurred in the face of greatly fluctuating soil moistures from saturation to less than Aw of 0.50 and soil temperatures of 17-25° C. (although the soil surface reached 50° C. in unshaded areas).

What is critical concentration of *Metarhizium* spores for SBRM control? MA1200 and F52 were subjected to multiple dose bioassay with 3rd instar SBRM larvae. Spores were mixed into quartz sand or field soil, to the desired concentration and 20% WHC. We observed linear concentration-percent mortality/infection responses. For 3rd instars, MA1200 spore concentrations of $>6 \times 10^6$ spores/cc soil were needed for >80% efficacy; F52 was more efficacious, achieving ~90% kill at 2.5×10^6 spores/cc soil. Field soils gave variable results. Presumably, 1st and 2nd instars need a smaller dose for lethal infection than 3rd instars. How does this translate to the field? A rate of 2.5×10^6 spores/cc soil in the top cm of soil profile equals 5×10^{13} /ha. This amount is about \$49/ha based on current prices of existing mycoinsecticides.

Does soil type affect the efficacy of *Metarhizium* spores? MA1200 conidia were mixed into five

soils, differing in sand:silt:clay ratios, at two moisture levels, 15% and 30% water holding capacity, to achieve 2.5×10^5 spores/cc soil. Soils were assayed with 3rd instar SBRM larvae; mortality was determined after three weeks. There were three replicates per assay and the assay was repeated twice. Efficacy ranged from 7-100% in the different soils at 15%WHC but 44-87% at 30%WHC, with the fungus responding differently to moisture in the different soils. CFUs remained unchanged within limits of precision during incubation.

Does soil moisture affect the infectivity of *Metarhizium* conidia in soil? Savage MT silt clay was inoculated with MA1200 conidia in sufficient water to achieve 10, 15, or 30% WHC. There were two doses: 2.5×10^5 and 2.5×10^6 CFU/g soil. Soils were assayed with 3rd instar SBRM larvae; mortality was determined after three weeks at 20-25° C. Infection/mortality by *Metarhizium* was proportional to moisture, decreasing to 0-11% at 10%WHC. The fungus seems much less effective in dry soil such as often exists in the top cm where sprays are applied.

Metarhizium vs. light insect pressure: In field trials (2002-2004) we applied *Metarhizium* granules at planting, using modified in-furrow application, and/or spores in a water based spray in 12-15 cm band-over-row in 187-374 L/ha (20-40 gpa) just as or just before flies moved into the fields, based on sticky trap observations. Fly pressure was light (<100 flies/trap total). In 2002, at Sidney, root damage was light but was reduced by *Metarhizium* to the level of terbufos; Yields were confounded by plant disease. In 2004, all fungus treatments caused a reduction in root damage equal to terbufos, and significantly different from the untreated control. There were no significant differences in yield, however, because the damage was very light.

Metarhizium vs. heavy insect pressure: In northeastern N.D. field trials fly numbers were much greater (>500 flies/trap). Treatments and design were similar to trials in Sidney MT. In 2003 *Metarhizium* did not reduce root damage and yields reflected damage. Terbufos afforded only slight protection. In 2004 fly pressure was >700 flies/trap. Root damage in terbufos plots was n.s.d. from untreated, reflecting severity of attack. Only combination of *Metarhizium* MA1200 granules and spray significantly reduced root damage, but yields were not significantly increased over control. Damage was too heavy. In both years, bioassays with 3rd instar larvae, of soil samples from *Metarhizium* spray-treated plots, gave high mortalities from mycosis – there was enough fungus in the top cm of soil for control. But plants were not protected from the heavy onslaught. The fungus did not work, at least by itself.

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