Control of Sugarbeet Root Maggot with the Fungus *Metarhizium anisopliae*

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ABSTRACT

Only a few insecticides are available for controlling sugarbeet root maggot (*Tetanops myopaeformis* von Röder). These could become less effective because of the development of resistant root maggot or become unavailable because of environmental concerns. Laboratory results suggested that the entomopathogenic fungus, *Metarhizium anisopliae* (Metschnikoff) Sorokin, had potential as a root maggot control agent and prompted the field testing described in this paper. *Metarhizium* inoculum was spread evenly over field plots in the fall preceding the sugarbeet (*Beta vulgaris* L.) crop, in the spring prior to planting, or both in the fall and spring. In 1995 trials at Hillsboro, North Dakota, plots not treated with insecticide yielded 32.6 Mg ha^{-1}, compared with 48.7 Mg ha^{-1} when a chemical insecticide was used. Root yields from the *Metarhizium* treatments ranged from 33.2 to 42.2 Mg ha^{-1}. Four-year (1996-99) average recoverable sugar yields at Crookston, Minnesota were 7161 kg ha^{-1} when no insecticide was applied, 8120 kg ha^{-1} when a chemical insecticide was used, and 8622 kg ha^{-1} when *Metarhizium* was applied in the spring and fall. Results, to-date, have been encouraging; however, information on application rates and timing, formulation, and the effectiveness of *Metarhizium* in more environments is required before commercialization is feasible.

Additional Key Words: *Beta vulgaris* L., biological control, entomopathogenic fungi, integrated pest management, *Tetanops myopaeformis* von Röder.

Root yield losses attributable to sugarbeet root maggot (*Tetanops myopaeformis* von Röder; Diptera: Otitidae) damage can be significant in a number of sugarbeet (*Beta vulgaris* L.) production areas (Yun and Sullivan,

1980; Blickenstaff et al., 1981), including the Red River Valley. In the absence of control measures, yield losses of 40% would be common in portions of Minnesota and eastern North Dakota (Campbell et al., 1998). The primary control method is the application of a granular insecticide at planting. Two organophosphate insecticides, terbufos and chlorpyrifos, are used extensively throughout the region (Dexter et al., 1998). This almost exclusive use of a few chemicals with similar mode of action is conducive to development of insecticide resistant root maggot. Organophosphate resistance has been confirmed in other insect species, including other Diptera (Bisset et al., 1990; Cilek et al., 1991). Alternative root maggot control strategies also would be required if current insecticides were no longer available because of environmental concerns or regulatory actions.

Metarhizium anisopliae (Metschnikoff) Sorokin, one of approximately 700 species of entomopathogenic fungi (Roberts, 1989), has received attention as a biological control agent in crops (Samson et al., 1994; Kaaya and Munyinyi, 1995; Rath et al., 1995; Booth and Shanks, 1998). Identifying characteristics and descriptions of *Metarhizium* are readily available (Humber, 1997), as are guidelines for isolating, propagating, and evaluating survival in the field (Goettel and Inglis, 1997). Environmental conditions favoring *Metarhizium* have been characterized (Walstad et al., 1970; Li and Holdom, 1995). The use of *Metarhizium* to control insects began in the late 1800s (Metchnikoff, 1879), or earlier. Interest in biological control solutions continued until the development of efficient chemical pesticides in the 1940s. Recent concerns regarding long-term effects of chemical pesticides have prompted renewed interest in biological control agents as components of integrated pest management schemes that may continue to include some chemical pesticides. Increased consumer demand for organically produced food also has stimulated development of biological control solutions to pest problems.

This report summarizes results from preliminary field evaluations of *M. anisopliae* as a biological control agent for sugarbeet root maggot. It encompasses one year of exploratory research at Hillsboro and Saint Thomas, North Dakota plus five years of more extensive trials at Hillsboro, North Dakota and Crookston, Minnesota.

MATERIALS AND METHODS

Field trials were located near Hillsboro, North Dakota, in 1995, and near Crookston, Minnesota, in 1996 through 1999. The experimental design was a randomized complete block with 4 to 6 replicates. Treatments were considered fixed effects and replicates random effects for the analysis of variance. The Least Significant Difference (LSD) procedure

was used to compare treatment means when the F-test for treatments was significant (α=0.10). The analysis was performed with the ANOVA procedure of the SAS software package.

Field plots consisted of 6 or 12 11-meter rows spaced 56 cm apart. Plots were planted with a commercial planter on May 19, 15, 5, 6, and 18 in 1995, 1996, 1997, 1998, and 1999, respectively, and thinned to 76,500 seedlings ha^{-1}. Weeds were controlled with herbicides, cultivation, and hand weeding. Cercospora leaf spot (caused by *Cercospora beticola* Sacc.) was controlled with fungicides when necessary.

The *M. anisopliae* applied in all the field trials was derived from a strain obtained from the American Type Culture Collection (No. 22099). This culture is native to Israel and was characterized as "virulent on larvae of many species". Mortality of third instar root maggot larvae exposed to this strain in the laboratory was 15, 94, and 100%, 7, 15, and 29 days after exposure, respectively. Mortality at 29 days for larvae not exposed to *Metarhizium* was 3% (Smith and Eide, 1995). The *Metarhizium* used in the field trials was isolated from infected root maggot larvae prior to large scale increase on autoclaved barley, to assure virulence to the maggot.

Autoclaved barley was inoculated with *M. anisopliae* to provide inoculum for field trials. To produce the inoculum, whole-grain barley was mixed with potato dextrose broth, autoclaved, and inoculated with conidia. The mixture was incubated at room temperature for 21 to 28 days in 2.8 L Fernbach flasks, and dried at 42 C for 5 days. The dried inoculum was spread evenly over the center six rows of field plots with a fertilizer spreader at a rate of 1.7 Mg ha^{-1} (per application) and incorporated with a field cultivator. To determine spore concentrations of the inoculum, 10 g samples of inoculated barley were placed in 100 ml of a 3% (volume/volume) Tween 80 solution, stirred for 30 minutes, and spores in the solution were counted with the aid of a hemacytometer. Spore (conidia) concentrations at the time of application ranged from 5.4×10^{13} to 9.1×10^{13} spores ha^{-1}. *Metarhizium* inoculum was checked periodically for infectivity on root maggot in the laboratory.

Inoculum for the 1995 sugarbeet crop was applied in the fall preceding the crop (27 September 1994), in the spring prior to planting (18 May 1995), or both in the fall and spring. The treatment application dates for the 1996 sugarbeet crop were 13 October 1995 and 14 May 1996. *Metarhizium* treatments for the 1997 sugarbeet crop consisted of fall (3 October 1996) and spring (5 May 1997) applications, a double rate (3.4 Mg inoculated barley ha^{-1}) spring application, and a fall plus spring application, all with an additional *Metarhizium* application (23 May 1996) prior to planting the barley crop preceding sugarbeet. Treatments for the 1998 and 1999 sugarbeet crops consisted of single and double rate fall applications,

single and double rate spring applications, and fall plus spring applications. Treatments were applied 21 October 1997 and 24 April 1998 for the 1998 sugarbeet crop and 3 November 1998 and 28 April 1999 for the 1999 crop. *Metarhizium* treatments were compared with chlorpyrifos (Lorsban) at 1.7 kg a.i. ha^{-1} banded over the row at planting and with no insecticide for maggot control. The barley inoculum was finely ground prior to application at Hillsboro in 1995; whole-grain barley was used in all other trials.

Root maggot damage was assessed in late July or early August each year. Damage ratings for individual plots were the mean of 10 roots rated on a 0 to 9 scale where 0 = no root maggot feeding scars; 1 = 1 to 4 small scars (pin head size); 2 = 5 to 10 small scars; 3 = up to 3 large scars or scattered small scars; 4 = a few large scars and/or numerous small scars; 5 = several large scars and/or heavy feeding on lateral roots; 6 = numerous scars with up to 25% of root scarred; 7 = 25 to 50% of root blackened by scars; 8 = 50 to75% of root blackened by scars; and 9 = more than 75% of root surface blackened. Roots were hand dug from the two rows adjacent to the rows that would be harvested later, washed, and immediately evaluated. All evaluations were based upon natural root maggot infestations at the site.

Harvest dates in 1995, 1996, 1998, 1999 and 1997 were September 22, 25, 23, and 23, and November 5, respectively. Plots at the Crookston site were defoliated with a mechanical defoliator and the center two rows were harvested with a commercial type harvester on the same day. A 10 to15 beet sample from each plot was sent to the American Crystal Sugar Company tare laboratory for sucrose and quality analysis. The Hillsboro plots were hand harvested, defoliated, and weighed.

RESULTS

Initial field studies with *Metarhizium* began in 1994. Inoculated barley was placed in the seed furrow along with the sugarbeet seed at Hillsboro and St. Thomas, North Dakota. The ratio of inoculated barley to sugarbeet seed was about 20 to 1 (by weight). At Hillsboro, the chlorpyrifos treatment increased root yield 12.1 Mg ha^{-1}, compared with the untreated check, but the yield of the *Metarhizium* treatment was not significantly better than the untreated check. Similar relationships were observed at St. Thomas where plots treated with chlorpyrifos yielded only 4.5 Mg ha^{-1} more than untreated plots.

Broadcast applications of *Metarhizium* were first examined in 1995 in a single trial near Hillsboro (Table 1). In this trial, the chlorpyrifos treatment produced 48.7 Mg ha^{-1}, compared with 32.6 Mg ha^{-1} for plots with no insecticide. When *Metarhizium* was applied, root yields ranged from 33.2

TABLE 1: Sugarbeet root maggot damage ratings and yield of sugarbeet treated with *Metarhizium*, Hillsboro, North Dakota and Crookston, Minnesota, 1995-1999.

			Metarhizium: application time and rate					No insecticide			
Location	Year	Lorsban	Fall + Spring	Spring double	Spring single	Fall double	Fall single	Untreated	Sterile barley	LSD	CV
		Damage rating (0 – 9) †									-%-
Hillsboro	1995	2.6 b*	3.9 a	- - -	3.9 a	- - -	4.2 a	4.2 a	- - -	0.7	14
Crookston	1996	3.0 d	2.9 d	- - -	3.6 b	- - -	3.4 c	4.2 a	- - -	0.2	4
Crookston	1997	3.6 d	4.0 c	4.4 ab	4.2 bc	- - -	4.6 a	4.6 a	- - -	0.2	6
Crookston	1998	1.8	1.8	1.8	2.0	1.9	1.7	1.3	1.6	ns	18
Crookston	1999	1.3	2.1	1.7	2.0	2.0	1.9	1.7	2.0	ns	24
		Root yield, Mg ha^{-1}									
Hillsboro	1995	48.7 a	42.2 b	- - -	37.5 bc	- - -	33.2 c	32.6 c	- - -	6.5	13
Crookston	1996	59.2 a	58.8 a	- - -	50.5 bc	- - -	51.5 b	49.7 c	- - -	1.4	3
Crookston	1997	57.6 a	57.5 a	56.5 a	51.4 b	- - -	52.6 b	48.7 c	- - -	2.7	5
Crookston	1998	50.4	52.7	51.8	51.5	49.7	48.1	48.1	48.1	ns	5
Crookston	1999	55.8 a	55.6a	55.3 a	51.2 c	52.4 bc	54.2 ab	51.0 c	48.0 d	2.4	4

* Means within a row followed by the same letter are not significantly different ($LSD_{0.10}$).

†Damage rating: 0 = no feeding scars; 1 = 1 to 4 small scars; 2 = 5 to 10 small scars; 3 = up to 3 large scars or numerous small scars; 4 = a few large scars and/or numerous small scars; 5 = several large scars and/or heavy feeding on laterals; 6 = numerous scars, up to 25% of root surface blackened; 7 = 25 to 50% of root blackened by scars; 8 = 50 to 75% of root blackened; and 9 = more than 75% of root surface blackened.

TABLE 1: (Continued) - Sugarbeet root maggot damage ratings and yield of sugarbeet treated with *Metarhizium*, Hillsboro, North Dakota and Crookston, Minnesota, 1995-1999.

			Metarhizium: application time and rate					No insecticide			
Location	Year	Lorsban	Fall + Spring	Spring double	Spring single	Fall double	Fall single	Untreated	Sterile barley	LSD	CV
					Sugar, g kg^{-1}						-%-
Crookston	1996	168	171	- - -	167	- - -	164	164	- - -	ns	5
Crookston	1997	151 b	158 a	146 c	150 bc	- - -	148 bc	151 b	- - -	4	3
Crookston	1998	170	180	179	178	176	184	176	180	ns	4
Crookston	1999	166	172	166	170	166	169	163	169	ns	5
					Recoverable sugar, kg ha^{-1}						
Crookston	1996	8685 a	9050 a	- - -	7596 b	- - -	7376 b	7124 b	- - -	703	8
Crookston	1997	7593 b	8043 a	7084 c	6773 cd	- - -	6782 cd	6425 d	- - -	371	5
Crookston	1998	7726 bc	8680 a	8267 abc	8321 ab	7730 bc	8016 bc	7628 c	7804 bc	643	6
Crookston	1999	8476 ab	8716 a	8595 ab	7911 bc	7954 bc	8314 ab	7470 c	7376 c	655	8

to 42.2 Mg ha^{-1} with the highest yields in plots receiving both a fall (1994) and spring (1995) application.

Application of *Metarhizium* in both fall and spring reduced root maggot damage ratings and produced root yields equal to a planting-time chlorpyrifos application in 1996 at Crookston (Table 1). When no insecticide was applied, root yields were 9.5 Mg ha^{-1} lower than when chlorpyrifos was used and 9.1 Mg ha^{-1} lower than when *Metarhizium* was applied in both spring and fall. A single *Metarhizium* application, either spring or fall, resulted in some reduction in root maggot damage and a corresponding root yield increase, but was inferior to the fall plus spring treatment. A measurable root yield response from the single fall application suggested that the introduced *Metarhizium* survived the winter of 1995-96.

The 1997 responses were similar to those observed in the 1996 trial. Root yield of the chlorpyrifos treated plots was 57.6 Mg ha^{-1}, compared with 48.7 Mg ha^{-1} from untreated plots. Plots receiving three *Metarhizium* (spring 1996 + fall 1996 + spring 1997) applications had root yields equal to the chlorpyrifos treated plots and all other *Metarhizium* treatments produced higher yields than the no insecticide treatment. The root yield produced by a spring 1996 + fall 1996 application indicated that *Metarhizium* remained effective over the winter of 1996-97 and provided some root maggot control in the 1997 sugarbeet crop. While our intent was to apply excess *Metarhizium* inoculum with each application, the nearly equal root yield from the spring 1996 + fall 1996 + spring 1997 and the spring 1996 + spring 1997-double rate treatments suggested that the reduced control obtained with the other two *Metarhizium* treatments (Table 1) could be a dosage effect and not a time of application effect.

Treatment effects on root maggot damage ratings and root yields were small in 1998. Although differences were not statistically significant, they appeared to follow trends observed in previous trials. Sterile barley (without *Metarhizium*) had no effect on root yield at Crookston in 1998. The spring of 1998 was extremely wet, resulting in saturated soils for extended periods. The effects of these conditions on *Metarhizium* survival and dispersal or on root maggot biology are not known. Root maggot damage was more severe and treatment effects more apparent in a nearby insecticide trial.

Although root damage ratings were low and differences among treatments were small in 1999, the chlorpyrifos treated plots yielded 4.8 Mg ha^{-1} more than the no-insecticide treatment. The fall + spring and the spring double-rate *Metarhizium* applications produced similar root yields,

again suggesting that time of application (fall or spring) is not as important as spore concentration. Root yields of the fall + spring, compared to the single-rate spring application, and fall applications, compared to no insecticide, implied at least some survival of *Metarhizium* over the winter of 1998-99.

Differences in sugar concentration were small and not statistically significant (α=0.10) in three of four years at Crookston. As a result, differences in recoverable sugar per hectare were primarily a reflection of root yield decreases associated with increased root maggot damage. In the year that significant differences were detected (1997), the response did not appear to be related to the amount of inoculum applied. The treatments that produced the highest sugar concentration (spring 1996 + fall 1996 + spring 1997) and the lowest sugar concentration (spring 1996 + spring 1997 - double rate) had similar root yields (57.5 and 56.5 Mg ha^{-1}, respectively) and received equal amounts of *Metarhizium* inoculum (applied in different sequences).

DISCUSSION

Year-to-year differences in the number of replicates and minor differences in treatments made a combined analysis over years inappropriate. In spite of this, consistently high relative recoverable sugar yield for the fall + spring application at Crookston and the relative root yields at Hillsboro (Table 1) indicate that *Metarhizium* has potential as a root maggot biological control agent.

Four-year average recoverable sugar yields at Crookston were 7161 kg ha^{-1} when no insecticide was applied, compared with 8120 kg ha^{-1} when chlorpyrifos was applied at planting and 8622 kg ha^{-1} when *Metarhizium* was applied in the fall and spring. Root maggot damage ratings for plots receiving no insecticide indicated that infestations were moderate to low for all trials. As a result, one can only speculate on the effectiveness of control with *Metarhizium* under severe root maggot infestations. During the years of this study, soil moisture was generally adequate, or in some cases surplus, for the crop. Moist soil generally is favorable for *Metarhizium* but it also may be favorable for some antagonistic organisms. Performance of *Metarhizium* under dry conditions similar to those encountered throughout the region in the late 1980s can not be predicted from results presented here.

Results presented in this report, observations from laboratory studies, and other field observations indicate *Metarhizium* merits further study as a possible sugarbeet root maggot biological control agent. The need for testing at more sites and under different conditions is recognized, but was

not feasible with the current inoculum production and application methods. Our trials were designed to confirm laboratory studies suggesting the potential of *Metarhizium* as a root maggot biological control agent. As such, they provide only limited information on application rates and timing. A formulation with a uniform concentration that could be applied with, at most, minor modification of conventional equipment would facilitate the extensive testing necessary for establishment of recommendations for commercial use.

Some commercial formulations (Schwarz, 1995; Moscardi, 1989; Booth and Shanks, 1998) may be available but the efficacy of these strains is not known. Even if commercial strains were found to be less effective than the strain used in these trials, the technology for producing commercial strains most likely could be adapted to other *Metarhizium* strains. We have tested a few *Metarhizium* strains in the laboratory and most appear to be effective against root maggot and superior to a commercial *Beauveria bassiana* (Balsamo) Vuillemin strain that was also examined.

Persistence in soil and consistency of control are important considerations with any biopesticide. The extent *Metarhizium* will increase or survive for extended periods under cultivation in the Red River Valley is not known. Reductions in root maggot damage and/or yield increases obtained from fall applications (Table 1), implied that at least some of the applied *Metarhizium* survived the winters of 1994-95, 1995-96, 1996-97, and 1998-99. *Metarhizium anisopliae* appeared to be more tolerant of agricultural disturbances than three other naturally occurring entomopathogenic fungi and was the only species found in sugarbeet fields in Finland (Vänninen, 1995). Although *Metarhizium* is capable of surviving on organic matter in the absence of a suitable insect host, the contribution of this saprotropic capability towards maintaining fungal populations in the field is not known. In some environments, other saprotropic microorganisms associated with soil and plant residues inhibit *Metarhizium* germination. In these situations, *Metarhizium* is restricted to insects for development (Walstad et al., 1970; Quintela and McCoy, 1998).

In a 1997 trial at Crookston, chlorpyrifos was mistakenly applied to five plots that had already received two or three *Metarhizium* applications. In this trial, plots treated with chlorpyrifos alone produced 53.5 Mg ha^{-1}, compared with 46.4 Mg ha^{-1} when no insecticide was applied. Plots receiving *Metarhizium* plus Chlorpyrifos averaged 59.6 Mg ha^{-1}, producing from 2.0 to 8.7 Mg ha^{-1} more than adjacent plots with chlorpyrifos alone. Conclusions based upon this limited information are very tenuous; however, the possibility that a combination of *Metarhizium* and chemical insecticide would enhance control has been suggested in other situations (Ferron, 1978). For example, combining *Metarhizium* or *Beauveria* with

imidacloprid (Gaucho) reduced the time to mortality of citrus root weevil (*Diaprepes abbreviatus* L.) in Florida (Quintela and McCoy, 1997).

Although biological control agents are generally considered to be safer than most chemical insecticides, they should not be handled recklessly. *Metarhizium* can cause eye irritation and the need for eye protection when handling inoculum became apparent early in our research program. While toxicity to healthy humans and other mammals is low, *Metarhizium* has been implicated as a complicating factor in individuals with malfunctioning immune systems (Burgner et al., 1998). The broad insect host range of many *Metarhizium* strains may be both a benefit, through control of other crop pests, and a detriment, in reducing populations of beneficial and other nontarget organisms (Genther and Middaugh, 1992). As with all pesticides and biological control agents, *Metarhizium* should be used judiciously and appropriate precautions should be exercised.

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