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INTEGRATION OF A FUNGAL ENTOMOPATHOGEN AND CEREAL COVER CROPS FOR SUGARBEET ROOT MAGGOT MANAGEMENT

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Introduction:

Sugarbeet Root Maggot (SBRM) *Tetanops myopaeformis* (Röder) is the most damaging pest of sugarbeet throughout the Red River Valley (RRV). It belongs to order Diptera and has three larval instars (Whitfield *et al.* 1984). Several authors (Gojmerac 1956, Theurer *et al.* 1982, Bechinski *et al.* 1989, and Campbell *et al.* 1998) have provided an estimate of SBRM damage, but the overall range of damage to sugarbeet crop can range between 9 to 85 percent within a season and average to about 40 percent. Currently, measures for controlling this insect pest are primarily focused on the chemical tactics and, to a limited extent, on cultural practices. Damage to the sugarbeet crop occurs extremely fast and often mandates the use of conventional insecticides. However, with changing technology and improved diagnostic tools, the use of biological control (biocontrol) measures is slowly gaining ground. Due to high toxicity and environmental side effects, many of the conventional insecticides used by sugarbeet growers are under review by the Environmental Protection Agency (EPA). The chief advantage of biocontrol programs incorporating entomopathogens (disease agents that are pathogenic to insect hosts) has been their high infectivity, host specificity, and environmental safety.

Since the early 1990s, *Metarhizium anisopliae* (Metschnikoff) Sorokin, a fungal entomopathogen has been under evaluation by USDA in the Red River Valley. Of several strains tested, strain MA-1200 has shown promise for use in bio-based SBRM control. However, variable results have been obtained. Appropriate formulation or delivery design has been an important aspect of *Metarhizium*-based biocontrol research programs, and many reports that evaluate *Metarhizium* formulations are currently available (Bartlett and Jaronski 1988, Periera and Roberts 1991, and Krueger *et al.* 1992). *Metarhizium* basically destroys SBRM larvae in four steps: attachment to the cuticle, penetration, multiplication in the hemocoel. Eventually, severe physical blockage and septicemia occurs due to network of hyphae in the host body and production of a complex of toxins, respectively.

Field evaluation of the cover crop strategy as a cultural method of SBRM control, however, started independently in the Sugarbeet Project of the NDSU Entomology Department in 1996. Carlson *et al.* (1997) evaluated cover crop combination with granular insecticides. They reported Lorsban 15G at 1.5 lb (AI)/ac and oat at 3.0 bu/ac to be the most effective treatment. Boetel *et al.* (2002) studied rye, oat, and barley at 0.5 and 1.5 OBE/ac (oat-bushel equivalent of seed per acre) in combination with various doses of Counter 15G. They reported Counter 15G at 1.5 lb (AI)/ac with rye at 1.5 OBE/ac to be the most effective treatment providing a yield of 6366 lb/ac of recoverable sugar.

The chief objective of this study, however, is to evaluate a combined approach to SBRM that integrates the microhabitat stability provided by a cover crop with different formulations of *Metarhizium anisopliae* MA-1200 for enhanced effectiveness. Overall, the study aims at developing a sustainable, cost-effective SBRM management strategy for growers in the Red River Valley of North Dakota and Minnesota.

Materials and methods:

This field experiment was established near St. Thomas (Pembina Co.) in North Dakota in 2002. A split-plot field design was used with two cereal cover crops (oat and rye) as main treatments, three seeding rates of 0, 1.5 and 3.0 oat bushel equivalent (OBE; this unit of measurement provides uniformity of plant stand based on the number of seeds in one bushel of oat) as the sub-treatments, and MA-1200 granular and liquid 2X formulations, Counter 15G@1.5 pound (AI)/ac, and no treatment as the sub-sub treatments. There were 20 treatments in the experiment (Table 1). Treatments were randomized at every level along with a check (no cover crop and no treatment). Plot length was 35 feet with six-rows of sugarbeet per plot. The planting date was 28 May, 2002. Oat variety used for this experiment was NEWDAK and the rye variety was DACOLD. Sugarbeet variety used was Beta 6600. Cereal cover crops were broadcast uniformly using the pop-bottle technique described be Boetel *et al.* (2002) just before planting sugarbeet. Planting-time granular *Metarhizium* and Counter formulations were applied modified-in-furrow (MIF). Soil temperature and moisture were monitored throughout the season using WatchDog® probes placed at planting depth (about 2 inches). *Metarhizium* foliar application was carried out about a month after planting and very close to the SBRM peak fly date, which occurred on June 28 this year. The foliar liquid application was applied in a total spray volume of 30 gallons/ac (GPA) using 8004E nozzles at 36 psi pressure in a 7-inch band. The cover crop was eventually killed by spraying SELECT herbicide at a rate of 8 oz/ac close to peak fly activity. After each *Metarhizium* application (granular or foliar), soil samples were immediately taken and analyzed quickly for the number of colony forming units (CFUs) in the laboratory on a selective growth medium (modified Veen's medium-glucose 10g, peptone 10g, ox gall 12 g, and agar 25g). Soil sampling was repeated once more for both *Metarhizium* treatments to assess survival under cover/no cover conditions. Plant stand counts were conducted twice: once after peak fly activity and another at harvest. The center 2 rows of each plot were harvested 30 September 2002 using a two-row modified commercial harvester. A mechanical defoliator was used to remove leaves from sugarbeets just prior to harvesting. Yield from each plot was individually recorded. Sugarbeet samples from each plot were sent to American Crystal Sugarbeet Tare Laboratory in East Grand Forks, Minnesota for chemical analysis. This analysis provided recoverable sucrose, % sucrose, and tare values for each sample.

Damage rating consisted of ten beets per plot from the outside two-rows of the treated rows. Damage Rating was done in late August. Blickenstaff *et al.* (1977) and Campbell *et al.* (1998) have demonstrated damage rating of sugarbeets to be good indicator of treatment success. We chose to use the 0 to 9 damage rating scale developed by Campbell *et al.* (1998) that has been in use at the Sugarbeet Project for several years (Boetel *et al.* 2001). We assessed root damage on ten beets per plot (five from row 2 and five from row 5 of each plot) in late August as indicator of treatment success in this experiment. Sugarbeets were dug, washed with water, and rated for damage as per the following rating scheme:

0: No scars
1: 1-4 small (pin head size) scars — Low Damage
2: 5-10 small scars
3: 3 large scars or scattered small scars
4: Few large scars and/or numerous small scars
5: Several large scars and/or heavy feeding on laterals — Moderate Damage
6: Up to ¼ root scarred
7: ¼-1/2 root blackened by scars
8: ½-3/4 root blackened by scars — High Damage
9: More than ¾ of root area blackened

Initially, the damage rating data was subjected to the GLM procedure. Damage rating data was subjected to analysis by contrasts using a SAS program (SAS Institute 1999). The contrast method of analysis (Steele and Torrie 1980) brought out several statistical differences among treatments. A probability (*P)* value less than 0.05 is considered significant (indicated in tables by *).

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Table 1. Treatment List for Field Study, St. Thomas, ND, 2002.

Cover crop	**Seeding rate (OBE)***	**Insecticide (chem. / bio.)**	**Treatment rate**	**Application timing**
OAT	1.5	Counter 15G	1.5# MIF**	Planting
OAT	1.5	Metarhizium	2X MIF	Planting
OAT	1.5	Metarhizium	2X spray	Postemergence
OAT	1.5	-----	----	----
OAT	3.0	Counter 15G	1.5# MIF	Planting
OAT	3.0	Metarhizium	2X MIF	Planting
OAT	3.0	Metarhizium	2X spray	Postemergence
OAT	3.0	-----	----	----
RYE	1.5	Counter 15G	1.5# MIF	Planting
RYE	1.5	Metarhizium	2X MIF	Planting
RYE	1.5	Metarhizium	2X spray	Postemergence

RYE	1.5	-----	----	----
RYE	3.0	Counter 15G	1.5# MIF	Planting
RYE	3.0	Metarhizium	2X MIF	Planting
RYE	3.0	Metarhizium	2X spray	Postemergence
RYE	3.0	-----	----	----
----	-----	Counter 15G	1.5# MIF	Planting
----	-----	Metarhizium	2X MIF	Planting
----	-----	Metarhizium	2X spray	Postemergence
----	-----	CHECK	----	----

*Oat Bushel Equivalent (1 OBE = same seeding density per unit area as 1 bushel of oat seed)
** Modified In-Furrow

Results and Discussion:

Oat Cover vs. Rye Cover Crop:

In Tables 2, 3, 4, and 5 the damage rating corresponds to treatment in the order of appearance.
This analysis is based on average damage ratings (Table 2). The results indicate that the granular treatment of *Metarhizium* was sensitive to cover crop type (contrast#1). The same interaction occurred when the two cover crops were contrasted without any treatment (contrast#3). However, the *Metarhizium* foliar treatment did not respond to cover crop type. Although statistically non-significant, rye with *Metarhizium* foliar performed numerically better than the oat combination (contrast #2). It is possible that a ground cover of rye by itself may be unfavorable for adult SBRM oviposition. Due to good ground cover *Metarhizium* was able to persist in soil prior to adhesion and infection of its host (SBRM).
Metarhizium granular had much greater root injury without a cover crop (Table 3).

Table 2. Contrasts of sugarbeet root maggot feeding injury with oat and rye treatment combinations, St. Thomas, ND, 2002

Contrast #	Contrast [a]	Root Injury Rating	F value	*P* value
1	Oat+MaG vs. Rye+MaG	5.72 vs. 4.67	9.62	0.0020*
2	Oat+MaF vs. Rye+MaF	5.58 vs. 5.01	2.50	0.1143
3	Oat+NT vs. Rye+NT	6.24 vs. 5.30	7.85	0.0052*
	Check	6.08		

[a] Notations:
MaG: *Metarhizium* granular planting time
MaF: *Metarhizium* foliar postemergent
Ctr: Counter @ 1.5lb (AI)/A
Oat1.5, Oat3.0: Seeding rates of oat cover crop
Rye1.5, Rye3.0: Seeding rates of rye cover crop
DR= Damage Rating
NT= No chemical/biological treatment

NC= No Cover Crop

Oat Cover vs. No Cover Crop:

Oat at 3.0 OBE/ac with granular *Metarhizium* provided significantly lower feeding injury than no cover granular *Metarhizium* combination (Contrast#2, Table 3). Root injury rating with oat 1.5 OBE/ac and granular Metarhizium was not significantly different from no cover granular *Metarhizium* treatment. *Metarhizium* spray with oat at 1.5 and 3.0 OBE/ac seeding rates did not show significant difference from *Metarhizium* with no cover treatment (Contrasts#3&4, Table 3). Thus in 2002 oat at high seeding rate showed good response with granular *Metarhizium* than at low seeding rate. This tells us that a dense canopy achieved by higher seeding rate of oat cover crop can provide good protection to *Metarhizium* granules (planting-time) applied in-furrow, probably by conserving soil moisture and lowering soil temperature. This integrated strategy may increase persistence and viability of *Metarhizium* spores in soil, resulting in lower SBRM feeding injury.

Table 3. Contrasts of sugarbeet root maggot feeding injury with oat and no cover treatment combinations, St. Thomas, ND, 2002

Contrast #	Contrast [a]	Root Injury Rating	F value	*P* value
1	Oat1.5+MaG vs. NC+MaG	5.98 vs. 6.70	2.35	0.1259
2	Oat3.0+MaG vs. NC+MaG	5.45 vs. 6.70	6.98	0.0084*
3	Oat1.5+MaF vs. NC+MaF	5.63 vs. 6.22	1.31	0.2533
4	Oat3.0+MaF vs. NC+MaF	5.53 vs. 6.22	1.79	0.1813
	Check	6.08		

[a] Notations:
MaG: *Metarhizium* granular planting time
MaF: *Metarhizium* foliar postemergent
Ctr: Counter @ 1.5lb (AI)/A
Oat1.5, Oat3.0: Seeding rates of oat cover crop
Rye1.5, Rye3.0: Seeding rates of rye cover crop
DR= Damage Rating
NT= No chemical/biological treatment
NC= No Cover Crop

Rye Cover Crop vs. No Cover Crop:

Three of the four contrasts of rye with *Metarhizium* (Contrast#1, 2, &4; Table 4) gave significantly lower root damage when compared to a no cover *Metarhizium* treatment. This set of contrasts with rye as cover crop clearly indicates the importance of an integrated approach for the success of a biocontrol based SBRM management program. As with oat, rye provides lower root injury with granular *Metarhizium*. This time however, both seeding rates of rye provided significant results. The foliar or spray application of the entomopathogen gave significantly different results than the no cover *Metarhizium* spray application only at a high seeding rate. These responses indicate that there is stronger interaction of biocontrol agent with rye cover crop, probably due to the dense crop canopy of rye that was evident in our field plots. It is too early to provide an interpretation linking the canopy effect with SBRM oviposition behavior. However, conservation of moisture and favorable soil temperature (by dampening effect) may be the main reasons for success of *Metarhizium* formulations.

Table 4. Contrasts of sugarbeet root maggot feeding injury with rye and no cover treatment combinations, St. Thomas, ND, 2002

Contrast #	Contrast [a]	Root Injury Rating	F value	*P* value
1	Rye1.5+MaG vs. NC+MaG	4.82 vs. 6.70	15.70	<0.0001*
2	Rye3.0+MaG vs. NC+MaG	4.52 vs. 6.70	21.13	<0.0001*
3	Rye1.5+MaF vs. NC+MaF	5.52 vs. 6.22	2.19	0.1394
4	Rye3.0+MaF vs. NC+MaF	4.50 vs. 6.22	13.29	0.0003*
	Check	6.08		

[a] Notations:
MaG: *Metarhizium* granular planting time
MaF: *Metarhizium* foliar postemergent
Ctr: Counter @ 1.5lb (AI)/A
Oat1.5, Oat3.0: Seeding rates of oat cover crop
Rye1.5, Rye3.0: Seeding rates of rye cover crop
DR= Damage Rating
NT= No chemical/biological treatment
NC= No Cover Crop

Conclusion and further research:

Main conclusions from 2002 field experiment are as follows:

- *Metarhizium* formulations perform better when integrated with cover crop
- More research is needed to design the best integrated approach for sustainable SBRM management using entomopathogens

This experiment suggests tritrophic interactions between treatments: cover crop (by providing modified soil microhabitat), the biocontrol agent (by improved persistence of *Metarhizium*), and the host (better control of SBRM) in sugarbeet cropping system. Further, retention of soil moisture and cooler temperature in the top soil may be keeping maggots in the treated zone causing higher mortality and protecting the sugarbeet tap root. However, currently only few reports suggest an association between cover crop and effects on entomopathogens (Lemanczyk and Sadowski 2002 provides a general account of the effects of oat cover crop on abundance of soil microorganisms). Therefore first-year research should be considered a trendsetter that will be reinvestigated in 2003 field season.

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