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INTEGRATION OF AN ENTOMOPATHOGENIC FUNGUS AND CEREAL COVER CROPS FOR SUGARBEET ROOT MAGGOT MANAGEMENT

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Introduction:

Sugarbeet is grown in about 13 US states and North Dakota is one of the leading states in production (Lange 1987). Sugarbeet is attacked by a number of insect pests that warrant the frequent use of chemical insecticides. In the sugarbeet cropping system, heavy reliance on organic synthetic insecticides has become a concern. Most of the popular organophosphates are available for restricted use or being phased out. Thus, it is necessary to find alternative control measures for root maggot control.
There are three major components of this project:

- SUGARBEET ROOT MAGGOT (SBRM), *TETANOPS MYOPAEFORMIS* (RÖDER)

This is the major insect pest of sugarbeet in the Red River Valley of North Dakota and Minnesota. The larval stage is the damaging stage that causes root scarring. Prevention of larval attack at an early growth stage of sugarbeet often mandates use of a planting-time insecticide. Yield loss potential from the root maggot has been reported to be 38% (Theurer et al. 1982) to 100% (Blickenstaff et al. 1981). The larvae can also use a wide range of host plants in the family Chenopodiaceae, besides sugarbeet (Bechinski et al. 1989). The larva uses mouth hooks to scrape the root surface and suck the oozing sap into its mouth chamber. Seedlings with damaged root tips can eventually shrivel and die. In the 2002 growing season, almost 70% of the total sugarbeet acreage was treated with chemical insecticides, primarily targeted to control SBRM (Luecke and Dexter 2003).

- *METARHIZIUM ANISOPLIAE* (METCHNIKOFF) SOROKIN

Metarhizium is an entomopathogenic fungus that produces characteristic green spores. The fungus infects a specific host in three phases: adhesion to insect body, penetration of outer body layer, and multiplication of fungus inside the body. Death of the insect occurs by a combination of mechanical stress, nutrient exhaustion, and toxins (Hajek and St. Leger 1994). All three larval stages of SBRM can be infected by the MA1200 strain of *M. anisopliae*. A “strain” of microbe describes its uniqueness of characters, including host specificity. MA1200 has been under evaluation by the United States Department of Agriculture (USDA) at Fargo, ND since 1996 (Smith et al. 1998). It has the advantages of being host-specific, compatible with other control methods, and environmentally safe. For the development of *Metarhizium* as a control option for growers, it is necessary to devise an appropriate application technology. High temperature, desiccation, and solar radiation are some of the common reasons for reduced survival of entomopathogens under field conditions (Carruthers et al. 1988).

- <u>CEREAL COVER CROPS</u>

Research on the root maggot control potential of cereal cover crops was initiated in 1996 at North Dakota State University (Carlson et al. 1997). In general, a moderate to high seeding rate of cover crops like oat, rye and barley, integrated with chemical insecticides, gave good control of SBRM (Armstrong et al. 1999, Boetel et al. 2002). Dregseth et al. (2003) reported the successful integration of oat at 1.75 and 3.0 bushel acre (bu/ac) with Counter 15G (terbufos).

The major objective of this project is to integrate cover crops with a fungal insect pathogen that can be adopted on a large scale by growers in Red River Valley for effective root maggot control. This project also aims at understanding the ecological factors that affect *Metarhizium* and finding a formulation that can be easily incorporated into current sugarbeet production systems.

Materials and methods:

This experiment was carried out at two separate locations at St.Thomas (Pembina Co., ND) in 2002 and 2003. Split-split-plot field design was used with oat and rye cover crops as main treatments. Sub-treatments were the three seeding rates: 0, 1.5, and 3.0 oat bushel equivalent per acre (OBE/ac; this unit of measurement provides uniform plant stand based on the number of seeds in one bushel of oat). MA1200 granular (at planting) formulation, MA1200 liquid (postemergence) formulation, Counter 15G@1.5 pound (AI)/ac, and untreated check served as sub-sub-level treatments. Thus, there were 20 treatment combinations (Table 1). Treatments were randomized at every level and compared to an untreated check (with no cover crop). Plot sizes were 35 feet by 22-inch row spacing (four middle rows of sugarbeet were treated). Sugarbeet variety VDH 66240, oat variety "Newdak" and the rye variety "Dacold" were planted in both years of this trial. Cereal cover crops were broadcast uniformly using the pop-bottle technique described by Boetel et al. (2002) immediately before planting sugarbeet and incorporated into the soil by using a walk-behind garden tiller. At-planting granular *Metarhizium* and Counter treatments were applied modified-in-furrow (MIF). Application rates were controlled using Noble metering units mounted on a commercial planter (John Deere 71 Flex). MIF applications prevent seeds from coming in direct contact with the insecticides. *Metarhizium* spray application was done about a month after planting, at peak fly activity (mid- to late- June). Postemergence sprays were applied in 7-inch bands in a total spray volume of 30 gallons/ac using 6503E nozzles at 33-psi pressure. Liquid and granular *Metarhizium* applications achieved a rate of $2x10^{13}$ infective spores per acre. The cover crops were killed by Poast® (sethoxydim) herbicide spray at the rate of 8 oz/ac when they were about 7 inches tall.

Damage ratings were done on ten beets from the two outer treated rows of each plot in August. We used the 0 to 9 damage rating scale developed by Campbell et al. (2000), which evaluates root scars as an indicator of treatment success. Beets were dug, washed, and rated for larval feeding injury as per the following points on the scale:

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

The damage rating data was subjected to the GLM procedure (SAS Institute 1999) and contrasts (Steele and Torrie 1980). *P*-values of less than 0.05 were considered significant (indicated in Tables 2 & 3 by *).

Results:

A. Summary of 2002 (Table 2, Majumdar et al. 2003):

- Check plot had a root injury level of 6.08 (indicated moderate pest pressure).
- Oat cover at 3 OBE/ac integrated with *Metarhizium* granules had significantly lower root scarring than its noncover counterpart.
- Rye at 1.5 and 3 OBE/ac integrated with *Metarhizium* granule at planting applications provided significantly less feeding injury than the use of pathogen in a nonintegrative approach.
- Rye at high seeding rate (3 OBE/ac) with *Metarhizium* post spray provided good control.
- Overall, our results indicated successful integration of *Metarhizium* granules with rye cover.
- In the absence of any control applications, rye cover provided better root protection than oat.

B. Year 2003 (Table 3)

- Check plots had a root injury level of 5.50 that indicated a moderate level of larval infestation.
- Oat at 1.5 and 3 OBE/ac integrated with post emergence *Metarhizium* sprays (in 7-inch bands) provided better root protection against maggot feeding than spray applications without a cover.
- Rye at 3 OBE/ac, combined with *Metarhizium* granules, had significantly less root scarring compared to noncover *Metarhizium* plots.
- The postemergence (liquid) formulation of *Metarhizium* was successfully integrated with both seed rates of rye. These treatment combinations were superior to no-cover plots.
- Counter 15G (terbufos) at 1.5 lb(AI)/ac treatments were significantly better than *Metarhizium* applications under nonintegrated approaches.
- Oat plots at both seeding rates and granular *Metarhizium* were not significantly better than corresponding noncover treatments.
- Granular and liquid applications of *Metarhizium*, combined with high and low seeding rates of oat, did not provide any significant reduction in root scarring.
- Integration of low-seeded rye with *Metarhizium* granular application was not significantly different from stand-alone treatment of granules.
- Stand-alone treatments of rye and oat were not statistically different from each other.
- Stand-alone *Metarhizium* granule and liquid (non-integrated) treatments gave similar results.

- Overall, combining planting-time granular and postemergence *Metarhizium* treatments with either oat or rye enhanced SBRM control.

Discussion:

Year 2003

Our data indicates that oat and rye can be integrated with granule or liquid formulations of *Metarhizium*. This may be due to one or more of the following reasons:

- Oat (at the high seeding rate) and rye (at the low seeding rate) provided adequate ground cover. Such ground cover cannot be provided by sugarbeets alone. Thus, the cover crops protected *Metarhizium* while it waits for its host (the SBRM larva).
- Rye has spreading type of growth due to multiple tillers and short internodes. Shade provided by rye may be modifying the microhabitat above the soil by maintaining optimum levels of soil moisture and temperature. Rye cover, at high seeding rates, efficiently filters the harmful ultraviolet radiations of sunlight (known to be harmful to fungi).
- High soil moisture may keep larvae near the soil surface that has been treated with the biological agent. Prolonged contact of *Metarhizium* spores with larvae increases infection. Also, the larvae may be food-stressed, making them more susceptible to *Metarhizium*. In some insects, food-stress has been reported to increase their susceptibility to entomopathogens (Hajek and St. Leger 1994). Under laboratory conditions, third-instar SBRM larvae become sluggish after infection. Under field conditions, fungal infection can drastically reduce ability of larvae to find beet root.
- The cover crops may also modify ability of the female flies to find beets for egg-deposition.
- *Metarhizium* infection in insects occurs in a series of steps (penetration to exiting from the host). This indicates the constantly adjusting nature of the fungus. Low humidity has been reported to be responsible for the failure of entomopathogens to begin infection (Hajek and St. Leger 1994). The success of this experiment indicates that *Metarhizium* is able to adapt itself to the presence of a cover crop while it waits for SBRM larva.
- Cover crops may provide a shelter for beneficial insects that may be above and/or below ground predators. The predator populations may be important modulators of SBRM population (Bechinski et al. 1989).
- Larvae may get infected in several repeat cycles of *Metarhizium* infection and aid in transmission of infection to uninfected larvae (cause slight increase of spores in soil).

Compiled Data

This two-year experiment clearly demonstrates the success of an integrated approach for root maggot control. Two major findings are:

- Under moderate pest pressure, integration of high-seeded rye with *Metarhizium* granules consistently gave superior root maggot control than nonintegrated approaches.
- Integration of rye cover at 3 OBE/ac with *Metarhizium* postemergence applications performed significantly better than its noncover counterpart.

Future research:

By laboratory studies, we will study the behavior of the root maggot larvae under conditions of biotic and abiotic stress (living and nonliving factors that modify larval behavior). Some insects such as grasshoppers and ants are known to avoid entomopathogens by changing their behavior. Behavioral adaptations have not been studied in case of root maggot larvae. Behavioral data may provide new clues about the life cycle of SBRM that can be helpful in designing biobased management programs for this key pest.

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Table 1. List of treatments for 2002 & 2003 field studies, St. Thomas, ND

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	----	----

Note:
*Oat Bushel Equivalent (1 OBE = same seeding density per unit area as 1 bushel of oat seed)
** Modified In-Furrow
# lb (AI)/ac
2X = 2 x 10^{13} spores/ac

Table 2. Significantly different contrasts of sugarbeet root maggot feeding injury from 2003 trial (St. Thomas, ND)

	Contrast #	Contrasts	Root Injury Rating[a]	F value	*P*-value
OAT + MA1200	1	Oat 3.0OBE + MA1200 Granule *Vs.* No cover + MA1200 Granule	5.45 *Vs.* 6.70	6.98	0.0084*
RYE + MA1200	2	Rye 1.5OBE + MA1200 Granule *Vs.* No cover + MA1200 Granule	4.82 *Vs.* 6.70	15.70	<0.0001*
	3	Rye 3.0OBE + MA1200 Granule *Vs.* No cover + MA1200 Granule	4.52 *Vs.* 6.70	21.13	<0.0001*
	4	Rye 1.5OBE + MA1200 Spray *Vs.* No cover + MA1200 Spray	5.52 *Vs.* 6.22	2.19	0.1394
	5	Rye 3.0OBE + MA1200 Spray *Vs.* No cover + MA1200 Spray	4.50 *Vs.* 6.22	13.29	0.0003*
OVERALL FOR GROUP	6	Oat + MA1200 Granule (overall) *Vs.* Rye + MA1200 Granule (overall)	5.72 *Vs.* 4.67	9.62	0.0020*
	7	Oat + MA1200 Spray (overall) *Vs.* Rye + MA1200 Spray (overall)	5.58 *Vs.* 5.01	2.50	0.1143
	8	Oat + No treatment *Vs.* Rye + No treatment	6.24 *Vs.* 5.30	7.85	0.0052*
	-	Untreated check	6.08	-	-

Note: MA1200 = *Metarhizium anisopliae*, OBE = Oat Bushel Equivalent per acre, [a] on 0 to 9 scale

Table 3. Contrasts of integrated and nonintegrated approaches for sugarbeet root maggot control, St. Thomas, ND, 2003

	Contrast #	Contrasts	Root Injury Rating[a]	F value	*P*-value
OAT + MA1200	1	Oat 1.5OBE + MA1200 Granule *Vs.* No cover + MA1200 Granule	5.02 *Vs.* 5.13	0.13	0.7216
	2	Oat 3.0OBE + MA1200 Granule *Vs.* No cover + MA1200 Granule	4.85 *Vs.* 5.13	0.96	0.3273
	3	Oat 1.5OBE + MA1200 Spray *Vs.* No cover + MA1200 Spray	4.90 *Vs.* 5.55	5.37	0.0208 *
	4	Oat 3.0OBE + MA1200 Spray *Vs.* No cover + MA1200 Spray	4.65 *Vs.* 5.55	10.29	0.0014 *
	5	Oat 1.5 OBE + MA1200 Granule *Vs.* Oat 1.5 OBE + MA1200 Spray	5.02 *Vs.* 4.90	0.20	0.6560
	6	Oat 3.0OBE + MA1200 Granule *Vs.* Oat 1.5OBE + MA1200 Granule	4.85 *Vs.* 5.02	0.39	0.5330
RYE + MA1200	7	Rye 1.5OBE + MA1200 Granule *Vs.* No cover + MA1200 Granule	4.90 *Vs.* 5.13	0.64	0.4228
	8	Rye 3.0OBE + MA1200 Granule *Vs..* No cover + MA1200 Granule	4.60 *Vs.* 5.13	3.50	0.0617 *
	9	Rye 1.5OBE + MA1200 Spray *Vs.* No cover + MA1200 Spray	4.98 *Vs.* 5.55	4.20	0.0407 *
	10	Rye 3.0OBE + MA1200 Spray *Vs.* No cover + MA1200 Spray	4.48 *Vs.* 5.55	14.68	0.0001 *
	11	Rye 3.0OBE + MA1200 Granule *Vs.* Rye 1.5OBE + MA1200 Granule	4.60 *Vs.* 4.90	1.14	0.2853
	12	Oat + MA1200 Granule (overall) *Vs.* Rye + MA1200 Granule (overall)	-	0.89	0.3449
	13	Oat + MA1200 Spray (overall) *Vs.* Rye + MA1200 Spray (overall)	-	0.06	0.8011

OVERALL FOR GROUP	14	Oat + No treatment (overall) *Vs.* Rye + No treatment (overall)	-	1.43	0.2316
	15	MA1200 Spray (overall) *Vs.* MA1200 Granule (overall)	-	0.01	0.9365
	16	MA1200 Spray (overall) *Vs.* No treatment (overall)	-	0.04	0.8421
	17	MA1200 Granule (overall) *Vs.* No treatment (overall)	-	0.08	0.7803
	18	MA1200 Granule (overall) *Vs.* Terbufos (Counter)	-	5.91	0.0153 *
	19	MA1200 Spray (overall) *Vs.* Terbufos (Counter)	-	6.30	0.0123 *
	-	Untreated check	5.50	-	-

Note: MA1200 = *Metarhizium anisopliae*, OBE = Oat Bushel Equivalent per acre, [a] on 0 to 9 scale