SOIL PERSISTENCE OF *METARHIZIUM ANISOPLIAE* APPLIED TO MANAGE SUGARBEET ROOT MAGGOT IN A COVER CROP MICROENVIRONMENT

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Abstract:

The sugarbeet root maggot, *Tetanops myopaeformis* (Röder), is a major insect pest of sugarbeet, *Beta vulgaris* L., in North Dakota, Minnesota, and Idaho. Three biocontrol field trials using the insect pathogen *Metarhizium anisopliae* (Metch.) Sorok. ATCC 62176 in conjunction with cover crops were conducted in 2002-2004. Granular and aqueous spray formulations of *M. anisopliae* were applied in furrow to replicated plots at 8 x 10^{12} viable conidia/ha. Oat *(Avena sativa* L.*)* and rye *(Secale cereale* L.*)* cover crops were planted prior to sugarbeet at three rates to create different microenvironments for the fungus. Soil samples were collected at 0, 30, or 60 d after treatment (DAT). Significantly higher numbers of conidia were detected in soil samples collected immediately after application in fungus spray plots compared to granule plots. This suggested delayed activation and proliferation of *M. anisopliae* conidia on granules, which has also been observed in the laboratory. Soil sampling and dilution plating results indicated a 90% decline in conidial viability for the aqueous formulation within 30 DAT. In 2002, a 1.5 to 7.7-fold increase in conidial density per gram of soil occurred between 0 and 60 DAT in plots treated with *M. anisopliae* granules. This increase was numerically higher in cover crop plots compared to non-cover plots. Soil moisture tension in cover crop plots was higher (i.e., average of 27 kPa) compared to no cover plots (17 kPa). It appears that granular formulations of *M. anisopliae* can persist in low soil moisture microenvironments that occur under a cover crop canopy. To our knowledge, this is the first report on the field persistence of *M. anisopliae* formulations when integrated with cover crops.

(**Key words:** sugarbeet root maggot, *Metarhizium anisopliae*, cover crops)

The sugarbeet root maggot (SBRM), *Tetanops myopaeformis* (Röder), is the most important insect pest of sugarbeet in the Red River Valley (RRV) of North Dakota and Minnesota, as well as the Snake River Valley of Idaho. Larvae of *T. myopaeformis* feed by scraping root surfaces and consuming the sap that exudes from feeding sites. Feeding injury can result in seedling death if the tap root becomes severed. Sugarbeet yield losses can reach 100% in the absence of control measures (Whitfield *et al.*, 1984). Alternative control methods would be critically needed if insecticide registrations were lost as a result of regulatory action or if SBRM populations developed insecticide resistance due to the chronic use of synthetic chemical insecticides for their control during the past few decades. Smith (1990) was the first to strongly advocate the development of a biocontrol program for SBRM management. Thereafter, Smith and Eide (1995) conducted laboratory assays with the entomopathogenic fungus *Metarhizium anisopliae* (Metch.) Sorok. isolate ATCC 22099, and reported high virulence to SBRM larvae. Campbell *et al.* (2006) tested the

pathogenicity of *M. anisopliae* on SBRM under field conditions and suggested conidia concentration, application timing, and soil moisture as the most important determinants for success in root maggot control. Those authors also suggested that commercial formulations should be designed for application with conventional equipment to facilitate adoption of fungus-based control tools by sugarbeet growers. Persistence and consistency of formulations also have been suggested as important features of a biopesticide for insect control in sugarbeet (Campbell *et al.*, 2000).

The issue of persistence of *M. anisopliae* conidia under natural conditions has been a topic of debate among insect pathologists. Clerk and Madelin (1965) suggested temperatures in the range of 8 to 25^{o}C as adequate for survival *M. anisopliae* conidia, and 45% relative humidity was suggested as optimal. Walstad *et al.* (1970) reported temperatures within a range of 15 to 35^{o}C and humidity exceeding 92% to be optimal for *M. anisopliae*. The optimum soil water activity (i.e., actual available water to fungal spores) for growth of *M. anisopliae* varies from 0.97 to 0.99 (Hallsworth and Magan 1999). Vänninen (1995) studied the effect of location, habitat, and soil type on the survival of entomopathogens under natural conditions, and reported that *M. anisopliae* conidia are capable of long-term survival and resistant to biodegradation in cultivated regions. Conidia were found nonviable if the soil temperature fell below 10^{o}C. Bing and Lewis (1993) and Hummel *et al.* (2002) found conservation tillage and no-till most conducive for persistence of *M. anisopliae* conidia in soil. Antagonistic microorganisms and solar radiation have been commonly cited as reasons for the failure of entomopathogens under field conditions (Braga *et al.*, 2001; Rangel *et al.*, 2004; Jaronski *et al.*, 2007).

Cover crops have been investigated for potential agronomic benefits in sugarbeet by several authors: seedling protection from wind damage (Fornstrom and Miller, 1996), improvement in soil stability (Sommer and Schwerdtle, 1984), and better retention of soil moisture (Fornstrom and Miller, 1996). The pest control potential of cover crops in sugarbeet has been mainly recognized in relation to either weed control (Fornstrom and Miller, 1996) or SBRM management (Dregseth *et al.*, 2003). Reasons for the success of an oat cover for root maggot control were speculated to be the conservation of soil moisture that could positively impact release of active ingredient from the granular formulation of a conventional insecticide (i.e., terbufos), and greater exposure of larvae to insecticides in the treated zone due to modified larval behavior in the microhabitat provided by the cover crop (Dregseth *et al.*, 2003). It is possible that cover crops could modulate the soil microenvironment for the benefit of an entomopathogenic fungus such as *M. anisopliae*. However, information regarding the persistence of *M. anisopliae* in the presence or absence of a cover crop has not been evaluated in sugarbeet. The objective of this research was to determine the effect of oat and rye cover crops on persistence of *M. anisopliae* conidia in the field.

Materials and Methods:

Field studies were conducted in 2002, 2003, and 2004 near St. Thomas (Pembina Co., ND), an area consistently infested with high root maggot populations. In the first two years, treatments were assigned to experimental units using a randomized complete block design (RCBD) with a split-plot arrangement. Cover crop seeding rate was the main-plot factor and three insecticide regimes and an untreated check plot were the subplot factors. In 2004, treatments were assigned to experimental units using an RCBD wih a split-split plot arrangement. Main-plots were cover crop type (i.e., oat or rye), subplots were seeding rate (0, 186, and 374 seeds/m^2), and sub-subplot factors were insecticidal treatments plus an untreated check. There were four replicates of each treatment combination in all study years.

Planting Methodology. Cover crop trials were established according to the procedures of Dregseth et al. (2003). Cultivars used were 'Newdak' oat, 'Dacold' rye, and Van der Have 66240 sugarbeet. In 2002 and 2003, oat was sown at 0, 186, and 233 seeds/m^2, and rye was sown at 0, 374, and 466 seeds/m^2. In 2004, the seeding rates for both cover crops were 0, 186, and 374 seeds/m^2. Plots were 10.7 m long by 3.3 m wide (six sugarbeet rows planted 0.56 m apart). The two outer rows of adjacent plots served as untreated guard rows. Cover crops were broadcast-sown in salt-shaker fashion by using clean 591-ml beverage containers with 1.5-cm or 2-cm diam. holes in the bottoms for delivery of rye and oat seed, respectively. Broadcast cover crop seed was immediately incorporated into soil by using a small walk-behind garden tiller. Sugarbeet was planted immediately after incorporation of cover crop seeds. Two applications of sethoxydim (Poast®, BASF Corporation, Research Triangle Park, NC) herbicide, at 0.22 and 0.45 kg (AI)/ha, were made one week apart to stop cover crop growth and ultimately kill the cereal plants when shoot length was about 15 cm.

Formulations of *M. anisopliae*. A granular formulation of *M. anisopliae* isolate ATCC 62176 was produced at the USDA-ARS Northern Plains Agricultural Research Laboratory (Sidney, MT). Fungus conidia were mass-produced using sequential, diphasic liquid-solid fermentation (Bradley *et al.*, 2002). Conidia of *M. anisopliae* produced on agar medium was used as primary inoculum for subculturing on liquid and then solid media. The liquid fermentation phase involved a fluid medium that was inoculated with the primary culture of *M. anisopliae*, incubated for 3 to 4 d at 25-26^{o}C. Liquid cultures containing blastospores were added to sterile hydrated barley (*Hordeum vulgare* L.) in sterile spawn bags for conidia production on solid substrate. This solid fermentation phase lasted for 8 d at 25$\pm$1^{o}C and constant darkness. Fungus conidia were harvested from the solid medium after slow drying for 7 d at 23-25^{o}C. Conidia were harvested by mechanical classification of the dreid whole solid culture to yield a powder having 5.6 x 10^{10} conidia/g. Conidial fractions of *M. anisopliae* were passed through 20- and 100-mesh screens to obtain a homogeneous powder. Viability, assessed by observing conidial germination on yeast extract benlate agar, was >95%. The dry conidia were coated onto corn grit (16/20-mesh) using 20% monosorbitan oleate (Tween 20, Sigma Chemical, St. Louis, MO) as a binder to produce the granular formulation with a titer of 3.6 x 10^{11} viable conidia/kg (approx. 2.5 x 10^5 conidia per granule). The corn grit was first coated with the binder by spraying the liquid with an airbrush and mixing within a V-cone blender. Then the requisite amount of conidia was dusted onto the slightly sticky carrier, and the mixture

vigorously blended in the blender. The formulation was stored under refrigeration until use. The formulation was prepared each year with freshly produced conidia.

The fungus was applied at 8.0 x 10^{12} viable conidia/ha in all study years. Treatments were applied to the four central rows of each six-row plot. The granular formulation was applied modified in-furrow (MIF) at planting in May. Modified in-furrow granules of *M. anisopliae* (22 kg/ha) and terbufos (1.68 kg [AI]/ha) were placed in the upper portion of the furrow, which minimized direct contact of insecticide and sugarbeet seeds. A commercial John Deere 71 Flex planter (Deere & Company, Moline, IL) equipped with Noble metering units (Remcor, Howe, TX) was used for application of granules at planting.

Conidia were directly suspended in a 0.1% monosorbitan oleate/water mixture for postemergence spray applications, which were made during peak SBRM fly activity in June of each year. The suspension of conidia was shaken vigorously immediately before and throughout applications to maintain a uniform suspension and ensure consistent delivery of the intended rate of conidia. Banded applications (10-15 cm) were made using a CO_2-propelled backpack sprayer or a tractor-mounted sprayer calibrated for an output volume of 280 L/ha. Sugarbeet seedlings were mostly in the 4- to 6-leaf stage at time of postemergence applications.

Recovery of *M. anisopliae* Conidia. Soil samples were collected at 0 d (i.e., immediately after treatment) and 30 or 60 d after treatment (DAT). A stainless steel core sampler (5 cm diam.) was used to sample to a 3.8-4.0 cm depth for monitoring fungus survival at cover crop sowing depth. For granular and spray formulations, two soil samples were collected from each of the outer two treated rows by carefully placing the soil corer on treated zone. The soil samples were deposited into clean Ziploc (Racine, WI) plastic bags and transported in a dry cooler from the field to the laboratory where they were stored at 5^{o}C pending laboratory processing.

The dilution plate method of Goettel and Inglis (1997) was used to assess viability of *M. anisopliae* conidia. Soil samples were mixed thoroughly before subsamples were drawn. Two subsamples, 1 g for dilution plating and 2 g for soil moisture estimation, were drawn from each composite sample. Soil samples for viability assays were suspended in 9 ml of sterile cold water containing 0.1% Tween (10^{-1} dilution). The soil suspension was sonicated for 15 minutes to break up soil clumps, then 1 ml of soil suspension was serially diluted to a 10^{-2} dilution. Aliquots (100 µl) were spread on four petri plates containing 20 ml of modified Chase medium (20 g oatmeal, 20 g agar, 0.6 g dodine, 1 ml Gentamycin, and 0.001 g crystal violet) (Chase *et al.*, 1996). Distinct green circular colonies of *M. anisopliae* formed on the blue medium. Colony forming units (CFUs) were counted after 10 d of incubation at 25^{o}C. The two-gram soil samples were dried at 65^{o}C for 48 h and reweighed to determine percent moisture. The CFU counts from dilution plates were adjusted to obtain final CFUs/g dry soil.

Monitoring Soil Temperature and Moisture. Soil temperature and soil water tension, an indicator of the availability of water in soil, were monitored continuously in cover crop plots. An untreated (i.e, no cover crop) plot also was monitored. WatchDog data loggers (Model 425, Spectrum Technologies, Inc., Plainfield, IL), placed inside radiation shields, were mounted on metal poles. A Watermark water tension sensor and a temperature probe were laid horizontally, positioned at seeding depth (i.e., 3-4 cm below soil surface), and fastened to the ground using U-shaped metal bolts. Calibrated sensors

recorded observations every 2 h and data were downloaded using SpecWare v. 6.0 software (Spectrum Technologies, Inc., Plainfield, IL) at the end of the growing season. Since the cover crop was at its best vegetative growth 15-29 d after planting (DAP), soil water potential and temperature data from data loggers were truncated to provide relevant information for that growth period to assess the impact of microhabitat on conidia survival. Weather data recorded by the North Dakota Agricultural Weather Network (NDAWN Center, North Dakota State University) for St. Thomas were obtained for reference.

Data Analyses. Mean CFU counts from each test year were subjected to analysis of variance (PROC ANOVA, SAS Institute 1999) using model appropriate for the design. In 2002 and 2003 the data were analyzed as an RCBD with a split plot in time arrangement. In 2004, the data were analyzed as an RCBD with a split-split plot in time arrangement. A folded *F*-test (Steel *et al.*, 1997) was conducted by using error mean sums of squares to determine feasibility of a combined analysis of 2002 and 2003 data because seeding rates were identical for those years. Treatment means from data sets having significant interaction terms were separated by using Fisher's protected least significant difference (LSD) test at $P = 0.05$.

Results:

Conidia Survival. Conidia counts from 2002-2004 are summarized in Table 1. Conidia of *M. anisopliae* survived at least 30 DAT in the field. Numerical increases in numbers of viable conidia were observed for the granular formulation of *M. anisopliae* between 0 and 60 DAT in 2002, although the increases were not significant. That trend was not confirmed in subsequent years because sampling was limited to 0 and 30 DAT. In general, numerically higher numbers of conidia were observed in spray plots (average from three trials = 1,386 CFUs/g dry soil, Table 1) than granule-treated plots (507 CFUs/g), irrespective of cover type and seeding rate; however, significant differences were not always detectable. Plots sown to a high seeding rate of rye had more viable conidia when the fungus was applied in a spray form rather than on granules. Irrespective of seeding rate, *M. anisopliae* conidia survived better in oat cover crop plots (mean = 1,104 CFUs/g) than in rye (mean = 765 CFUs/g) or non-cover (mean = 992 CFUs/g) plots.

The analysis of variance (ANOVA) indicated that 2002 and 2003 data could not be combined because the error mean squares from the folded test were significantly different from each other. Therefore, results from the ANOVA are provided separately by year in Table 2. The ANOVA for 2004 persistence data is provided in Table 3. In 2002 and 2003, there was no significant effect ($P > 0.05$) of cover crops on the survival of *M. anisopliae* conidia. There also was no effect of either the cover crop type or seeding rate ($P > 0.05$) on conidial viability in 2004.

Table 1. Conidia counts of *M. anisopliae* applied as granules (MaG) or aqueous spray (MaS) formulations combined with cover crops to manage *Tetanops myopaeformis*, St. Thomas, ND, 2002-2004

	CFUs/g dry soil ± SD		
Treatment[a]	**0 DAT**	**30 DAT**	**60 DAT**
2002			
Oat186 + MaG	247 ± 102		1902 ± 1956
Oat186 + MaS	8285 ± 4515	1527 ± 345	
Oat233 + MaG	530 ± 481		905 ± 672
Oat233 + MaS	10867 ± 1663	1467 ± 1118	
Rye374 + MaG	467 ± 560		1090 ± 1164
Rye374 + MaS	11247 ± 6764	872 ± 838	
Rye466 + MaG	622 ± 394		1215 ± 873
Rye466 + MaS	16430 ± 11481	1527 ± 1151	
MaG	1185 ± 713		1875 ± 1702
MaS	10310 ± 4466	1652 ± 1093	
2003			
Oat186 + MaG	62 ± 125	0	NA[b]
Oat186 + MaS	1580 ± 747	372 ± 528	
Oat233 + MaG	125 ± 250	0	
Oat233 + MaS	4092 ± 3775	247 ± 339	
Rye374 + MaG	92 ± 119	0	
Rye374 + MaS	3182 ± 1599	155 ± 120	
Rye466 + MaG	60 ± 69	30 ± 60	
Rye466 + MaS	4530 ± 3456	560 ± 678	
MaG	62 ± 125	0	
MaS	2560 ± 331	217 ± 257	
2004			
Oat186 + MaG	122 ± 174	92 ± 185	NA[b]
Oat186 + MaS	58800 ± 40984	4465 ± 1771	
Oat374 + MaG	122 ± 174	0	
Oat374 + MaS	46300 ± 9998	2277 ± 1888	
Rye186 + MaG	155 ± 237	62 ± 125	
Rye186 + MaS	55800 ± 17967	1655 ± 1647	
Rye374 + MaG	312 ± 473	0	
Rye374 + MaS	55750 ± 11800	2030 ± 385	
MaG	92 ± 185	437 ± 718	
MaS	49175 ± 15575	1777 ± 1402	

DAT = days after treatment.

[a] Numbers indicate seeds per m^2 of the cover crops.

[b] Not available.

Table 2. Factorial analysis of variance to evaluate effects of cover crop type, fungus treatment (formulation), and sampling date on *Metarhizium anisopliae* spore persistence, St. Thomas, ND, 2002-2003

		2002			2003		
Source	**df**	**Mean square**	***F***	***P***	**Mean square**	***F***	***P***
Replication	3	1051.809	0.95	0.4286	219.123	1.38	0.2693
Cover type[a]	4	884.037	0.74	0.5850	159.059	1.24	0.3474
Error (a) (Replication x Cover type)	12	1201.300	-	-	128.788	-	-
Formulation[b]	1	58639.035	45.41	<0.0001	5824.284	45.93	<0.0001
Cover type x Formulation	4	1045.222	0.81	0.5383	149.918	1.18	0.3583
Error (b) (Replication x Cover type x Formulation)	15	1291.292	-	-	126.811	-	-
Sampling date[c]	1	42610.296	31.87	0.0110	4360.104	17.25	0.0254
Error (c) (Replication x Sampling date)	3	1337.127	-	-	252.688	-	-
Cover type x Sampling date	4	1118.789	1.01	0.4174	132.279	0.83	0.5150
Formulation x Sampling date	1	58379.415	52.94	<0.0001	3931.208	24.81	<0.0001
Cover type x Formulation x Sampling date	4	773.560	0.70	0.5977	129.339	0.82	0.5260
Error (d)	27	1102.7245	-	-	158.462	-	-

[a] Oat was sown at 0, 186, and 233 seeds/m^2 and rye was planted at 0, 374, and 466 seeds/m^2.
[b] Conidia of *M. anisopliae* were applied as granules or aqueous sprays.
[c] Samples were taken at 0 and 30 or 60 d after treatment.

Table 3. Analysis of variance to evaluate the effects of cover crop type, seeding rate, fungus formulation, and sampling date on persistence of *Metarhizium anisopliae*, St. Thomas, ND, 2004

	2004			
Source	**df**	**Mean square**	***F***	***P***
Replication	3	16791.945	1.39	0.2682
Cover type[a]	1	83.364	0.01	0.9152
Error (a) (Replication x Cover type)	3	6226.715	-	-
Seeding rate[b]	2	4552.400	0.35	0.7169
Cover type x Seeding rate	1	7070.494	0.54	0.4826
Error (b) (Replication x Cover type x Seeding rate)	9	13180.971	-	-
Formulation[c]	1	1387151.420	145.50	<0.0001
Cover type x Formulation	1	573.018	0.06	0.8097
Seeding rate x Formulation	2	3449.191	0.36	0.7023
Cover type x Seeding rate x Formulation	1	3990.279	0.42	0.5274
Error (c) (Replication x Cover type x Seeding rate x Formulation)	15	11073.077	-	-
Sampling date[d]	1	1093204.911	70.67	0.0035
Error (d) (Replication x Sampling date)	3	15468.986	-	-
Cover type x Sampling date	1	1545.637	0.13	0.7235
Seeding rate x Sampling date	2	3509.930	0.29	0.7503
Formulation x Sampling date	1	1222780.369	101.18	<0.0001
Error (e)	26	12085.752	-	-

[a] Oat or rye were planted in plots.
[b] Oat was sown at 0, 186, and 233 seeds/m^2, and rye was planted at 0, 374, and 466 seeds/m^2.
[c] Conidia of *M. anisopliae* were applied as granules or aqueous spray.
[d] Samples were taken at 0 and 30 or 60 d after treatment.

Significant factors affecting conidia viability in 2002, 2003, and 2004 were *M. anisopliae* formulation and sampling date (Tables 2 and 3). Sampling date and fungus formulations together had a major impact on conidia persistence, as was indicated by the significant formulation x sampling date interaction for 2002 ($P < 0.0001$), 2003 ($P < 0.0001$), and 2004 ($P < 0.0001$). Conidia count data were pooled for formulation and sampling date and the final counts have been graphically depicted in Figure 1).

Effect of Cover Crops on Soil Microclimate. Soil water tension at sugarbeet seeding depth (i.e., 3-4 cm) was moderated by the cover crop type and seeding rate (Table 4). In 2002 and 2003, plots seeded with the high rate of oat had higher water tension (29 and 60 kPa, respectively) than the water tension (19 kPa) observed in plots seeded with the low rate. Oat plots seeded at the high rate, thus, had much drier soil than those plots at the lower seeding rates. A higher seeding rate of rye also was associated with higher water tension. In 2004, the low seed rate of oat and rye had high water tension. Weather varied from year to year within the study (Table 5). Rainfall observations indicated that May was the driest month (i.e., only 46 mm rainfall) in 2002, whereas rainfall amounts in May of 2003 and 2004 (i.e., 95 and 91 mm, respectively) were much higher. In 2002, the most rainfall (i.e., 132 mm) was received in June.

Table 4. Impact of living cover crops on soil temperature and soil water potential 15-29 d after planting, St. Thomas, ND, 2002-2004

	2002[a]		2003[a]		2004[b]	
Cover type and seeding rate	**Soil temperature (°C)**	**Soil water tension (kPa)**	**Soil temperature (°C)**	**Soil water tension (kPa)**	**Soil temperature (°C)**	**Soil water tension (kPa)**
Oat, low rate	23	19	17	19	16	31
Oat, high rate	23	60	17	29	24	19
Rye, low rate	23	18	15	12	20	31
Rye, high rate	23	21	NA[d]	28	16	28
No cover	21[c]	NA[d]	15	17	16[c]	NA[d]

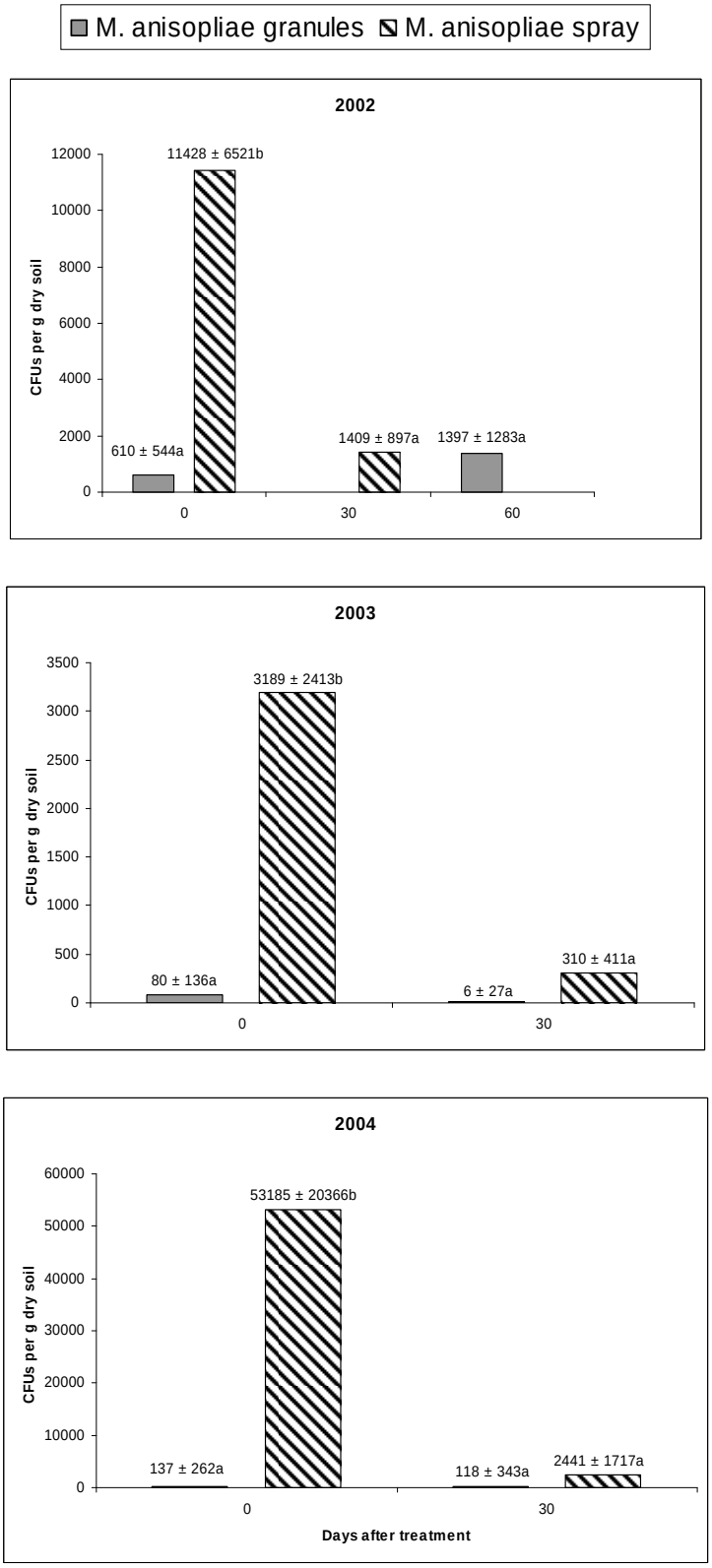
[a] Seeding rate of oat: 0, 186 (low rate), and 233 seeds/m^2 (high rate); seeding rate of rye: 0, 374 (low rate), and 466 seeds/m^2 (high rate).
[b] Seeding rates of oat and rye were the same, i.e., 0, 186 (low rate), 374 (high rate) seeds/m^2.
[c] Source: NDAWN (2006); temperature at 10 cm depth.
[d] Data not available due to sensor malfunction.

Table 5. General weather information during the sugarbeet production season at St. Thomas, ND, 2002-2004

Year	**Month**	**Soil temperature (°C) at 10 cm depth**	**Solar radiation (MJ/m^2)**	**Rainfall (mm)**
2002	May	8	22	46
	June	20	22	132
2003	May	13	18	95
	June	20	22	72
2004	May	12	17	91
	June	17	21	20

[a] Source: NDAWN (2006).

Fig. 1. Colony forming units of *Metarhizium anisopliae* per gram of dry soil (mean ± SD) at 0, and 30 or 60 d after treatment with a granular or liquid application of the fungus at St. Thomas, ND, 2002-2004. Means within a year followed by the same letter are not significantly different from each other (LSD, α = 0.05).



Discussion:

Significant formulation x sampling date interactions occurred in all field trials; thus, indicating that *M. anisopliae* formulation, delivery technique, and application timing can affect the success of this fungus as a SBRM biocontrol agent. There was no evidence of increased persistence of *Metarhizium* conidia in cover-cropped plots. At the seeding rates tested, the cover crops did not appear to impact conidia persistence, but oat plots generally had higher numbers (mean = 1,104 CFUs/g) of viable conidia at 30 DAT than rye plots. Cover crops also appeared to impact soil moisture. Higher cover crop seeding rates dried the soil more than the low seeding rate. Results indicate resiliency of *M. anisopliae* conidia and appear to match the observations of Vänninen (1995) and Hummel *et al.* (2002). In the present study, *M. anisopliae* conidia persisted in soil for at least four weeks at temperatures ranging between 13 and 26^{o}C and soil moisture ranging from 19-60 kPa (Table 4). The findings relating to soil temperature impacts on survival of *M. anisopliae* conidia agree with those of Clerk and Madelin (1965).

The two methods for delivery of *M. anisopliae* conidia resulted in some interesting trends. Conidia counts increased in plots treated with fungus granules when soil samples were collected at 60 DAT compared to 0 and 30 DAT in 2002. This suggests the possibility that some degree of sporulation and multiplication could have occurred in soil. Such increases would not likely contribute substantially to the total concentration of conidia in soil. However, this finding implies the likelihood of conidial persistence for up to 60 DAT when the fungus is applied via modified in-furrow placement to soil as a planting-time granule. Fungus conidia coated onto a corn-based carrier could require a latency period after application for release and infectivity. Moisture is also crucial for germination of fungus spores in soil (Walstad *et al.*, 1970). In this context, the *M. anisopliae* granule acted similar to conventional insecticides that also typically have a latency period until the toxicant is activated by soil moisture. Therefore, the application of *M. anisopliae* formulations should be timed appropriately to synchronize infectivity with the presence of a susceptible stage of the host. Low and infrequent rainfall events, such as those of 2002, could result in reduced soil moisture and delayed germination of *M. anisopliae* conidia on granules.

Conidial survival in plots treated with *M. anisopliae* sprays dropped dramatically between 0- and 30-DAT. The loss of viable conidia in spray plots averaged 91% in this study. Conidia survival in granule plots decreased by 52% during the shorter (30 DAT) sampling intervals in 2003 and 2004, although this was not a statistically significant decline. Survival and proliferation of conidia applied via the granular formulation could have resulted from the nutritive properties of the granular carrier (i.e., corn-grit substrate). Placement of fungus conidia in a manner that provides protection from the detrimental effects of solar radiation, such as the modified in-furrow placement of granules in this study, appears to prolong the field efficacy of biocontrol fungi.

The findings from this experiment suggest that cereal cover crops could be useful in extending the activity period of biocontrol fungi such as *M. anisopliae* isolate ATCC 62176 by improving the soil microclimate for fungal survival and proliferation. Synchronizing the fungus with the presence of susceptible insects will also be an important factor for increasing the likelihood of successful insect control. Although not a component of this study, cover crops appeared to reduce microsite wind velocity near the soil surface (A.M., *personal observation*). This also could be a reason for the effectiveness of cover crops as reported by Fornstrom and Miller (1996). Reduced wind velocity could minimize loss of soil moisture by evaporation. The

net effect of a ground cover could be beneficial to a fungus such as *M. anisopliae*, especially in production seasons characterized by extended periods of low rainfall. A key contributor to soil moisture in dryland crop production, rainfall, also greatly influences soil temperature due to evaporative cooling. Dry periods following heavy rainfall were tolerated by conidia, but such environmental stressors could potentially cause treatment failure. Therefore, formulation and possibly strain (i.e., drought- and/or heat-tolerant) are likely to play major roles in the commercial feasibility of this organism for SBRM control under such variable environmental conditions.

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