Persistence and movement of fungal conidia applied to soil for the management of the sugarbeet root maggot, *Tetanops myopaeformis* (Röder).

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Abstract

The fungus, *Metarhizium anisopliae* (Metschnikoff) Sorokin, has potential as an alternative to chemical soil insecticides for the management of the sugarbeet root maggot. For 3 years (1996-98) in the same field, soil applications of an isolate of the fungus (ARS-T1) were in a rotation of wheat, barley, and sugarbeets and seasons (fall and spring) to evaluate the effectiveness of management strategies. There is a need to better understand what happens to the fungus in the soil after applications (i.e., what concentration of conidia is present, do conidia persist throughout the season, does the fungal concentration build up with repeated applications or timing of applications, and do conidia move within the soil profile?). From May 27 to Aug 19 of 1998, soil samples within the top 22.5 cm (in increments of 7.5 cm) of the soil profile were taken every 2 weeks and analyzed for the presence and quantity of conidia. Concentrations of conidia for the fungal treatments ranged from $\approx 4.0 \times 10^4$--1.6×10^5 CFU/g of soil throughout the sampling period. The number of conidia did not increase with more applications, nor did timing of applications (fall vs. spring) affect the levels of conidia present in the soil. 57--89% of the conidia was present in the top 7.5 cm of the soil profile, and there were significantly fewer conidia present in the middle and bottom 7.5 cm of the soil profile when applications were made for only 2 years. The data suggest the fungus persists overwinter and during the period of maggot activity with no appreciable reduction in conidial concentration, there is minimal buildup of inoculum over the years or with timing of applications, and there is more downward movement of conidia with more years of applications.

Introduction

Alternatives to chemical soil insecticides for the management of the sugarbeet root maggot, *Tetanops myopaeformis* (Röder), are currently being investigated. The fungus, *Metarhizium anisopliae* (Metschnikoff) Sorokin, has a demonstrated potential in managing soil insect pests (Rath et al. 1995, Schwarz 1995). In particular, a strain of this fungus (ARS-T1) is pathogenic for the sugarbeet maggot, and soil applications in experimental plots of ARS-T1 has reduced damage caused by the maggot and increased sugarbeet yield when compared with untreated plots (Campbell et al. 1999). An understanding of what happens to the fungus after application is critical to success of its use. Some key questions that need to be answered are: 1) what concentration is present after application, 2) does the fungus persist throughout the period during which the root maggot is active, 3) does the inoculum concentration increase over time with repeated applications, 4) does timing of applications (fall vs spring) affect inoculum concentration, and 5) do conidia move within the soil profile?

Materials and Methods

The experiment was conducted on the University of Minnesota Northwest Experiment Station near Crookston, Minnesota. From 1996--1998, the 3 x 11-m treatment plots (each separated by 3 m) were planted in a crop rotation consisting of wheat in 1996, barley in 1997, and sugarbeets

in 1998. The 7 experimental treatments consisted of soil applications of a fungal strain that varied by timing (fall vs spring) and number of applications (Table 1); the experimental design was a randomized block with 6 replications that were separated by 6 m. The *M. anisopliae* strain, 22099, used in this research was obtained from the American Type Culture Collection. The strain was re-isolated from 3rd-instar root maggot, redesignated as ARS-T1, and maintained on potato dextrose agar. For field applications, the strain was produced on autoclaved barley seeds presoaked in 1% potato dextrose broth and dried at 42°C. In all 3 yr, 6.8 kg of ARS-T1 on barley ($\approx 5 \times 10^{13}$ -- 7×10^{13} conidia per ha) were broadcast using lawn fertilizer spreaders and incorporated with a field cultivator. But, the number of viable conidia applied per 6.8 kg and dates of application varied over the years, respectively--Spring 1996, no counts were made, May 23; Spring 1997, 1.9×10^{11}, May 6; Fall 1997, 1.2×10^{11}, October 21; and Spring 1998, 2.4×10^{11}, April 21. The soil was a loam soil with 46% sand, 34% silt, 20% clay, 4% organic matter, a pH of 8.1, and a bulk density of 1.17 g/cm^3.

Table 1. Listing of treatments of applications of ARS-T1 from 1996--98

Treatments	
1. S6S7S8	S = Spring treated
2. S6S7F7	F = Fall treated
3. S6S7FS8	U = Untreated
4. U6S7S8	6, 7, & 8 = Calendar year 1996, 1997, & 1998
5. U6S7F7	
6. U6S7FS8	
7. U6U7U8	

Three soil cores (1.88 cm diameter x 22.5 cm deep) were taken every 2 wk in each treatment plot from May 28 to August 19, 1998. From each 22.5-cm soil core, 3 subsamples were cut into 7.5-cm increments corresponding with the top, middle, & bottom 7.5 cm and placed in plastic bags. In the laboratory, each soil sample was placed into a paper bag and air-dried from 3--7 d. The sample was then pulverized with a mortar and pestle. To 90 ml of autoclaved distilled water, 10 g of soil was added and mixed for 30 min on a magnetic stirrer. A series of three 10x dilutions were made from which a 0.5-ml aliquot was pipetted from each and spread over a selective medium (Liu et al. 1993) in a 100 x 15 mm petri dish. The petri dishes were held at 25°C in the dark and the number of CFU (colony forming units) were counted after ≈3 wk. Data (# of CFU/g of soil) were analyzed by analysis of variance (ANOVA) as a repeated measure for each 7.5-cm increment and treatments were separated by LSD ($P = 0.05$).

Results

In the top 7.5 cm of the soil profile, the number of CFU/g of soil fluctuated throughout the sampling period and ranged from $\approx 4.0 \times 10^4$--1.6×10^5 CFU/g among the fungal treatments. These concentrations represent ≈57--89% of the conidia isolated from the top 22.5 cm of the soil profile. The number of CFU/g of soil decreased with increased depth. The number of CFU/g of soil in the middle and bottom 7.5 cm ranged from $\approx 2.0 \times 10^3$ --7.8×10^4 and 1.0×10^3--2.1×10^4, respectively. The concentrations of conidia for all fungal treatments within all 3 soil increments did not decrease throughout the period of sampling of 80+ d. During the 2 and 3 yr of fungal applications, the

experimental plots received 0, 2, 3, or 4 applications of ARS-T1 (Table 2). The mean number of CFU/g of soil for the entire sampling period did not significantly increase with more applications nor did timing of applications (fall vs. spring) affect the concentration of conidia present in the top 7.5 cm of the soil profile. In the middle and bottom 7.5 cm, the mean number of CFU/g of soil did significantly increase after 3 yr of applications compared to 2 yr. *M. anisopliae* was isolated from plots in which no fungal applications were made; the number of CFU/gram of soil averaged 2.2 x 10^3, 4.4 x 10^2, and 2.5 x 10^2 in the top, middle, and bottom 7.5 cm, respectively.

Table 2. Mean number of CFU/g of soil (x1000) among experimental treatments for the entire sampling period from May 27--August 19, 1998 in top, middle, and bottom 7.5 cm of a 22.5-cm soil core

		Mean # of CFU/g of soil (x1000)		
Treatments[1]	# of applications	Top 7.5 cm	Middle 7.5 cm	Bottom 7.5 cm
S6S7S8	3	102.81	36.38	20.86
S6S7F7	3	55.62	20.80	18.67
S6S7FS8	4	78.00	18.32	16.16
U6S7S8	2	82.62	9.60	3.41
U6S7F7	2	96.57	7.43	3.39
U6S7FS8	3	111.10	4.56	2.94
U6U7U8	0	2.21	0.44	0.25
LSD (.05)		22.25	7.34	5.43

[1]ARS-T1 applied: S = Spring; F = Fall; U = Untreated; 6, 7, & 8 = Calendar year 1996, 1997, & 1998.

Discussion

A critical aspect of the use of any insect pathogen such as a *M. anisopliae* is to establish and maintain a concentration of conidia that effectively manages the target pest population. Our results indicated the maximum concentration of conidia (≈10^5 CFU/g of soil) within the top 22.5 cm of the soil profile that could be expected from similar multiple applications of ARS-T1. Also, concentrations in the top 7.5 cm were not increased with more applications over years or within the same growing season, possibly a 10-fold increase in application rates is necessary to show a significant increase in concentration. A concentration of 10^6 conidia/g of soil has been suggested as necessary for acceptable pest mortality (Schwarz 1995), however, this concentration can change based on target pest and life stage. For example, Rath et al (1995) reported an 82% reduction in survival of 3rd-instar *Adoryphorus couloni* with *M. anisopliae* concentrations of ≈5 x 10^4 CFU/g in the top 10 cm of soil. The concentrations of ARS-T1 necessary to cause mortality in the sugarbeet maggot's instars are not known; identifying these effective concentrations will be a priority of future

laboratory research. Relative to persistence once established, the inoculum concentrations of ARS-T1 were maintained throughout the period of maggot activity from June to August. Fungal persistence is enhanced in the soil, and previous research demonstrated fungi can persist in the soil for months or longer (Gaugler et al. 1989, Samson et al. 1994, Rath et al. 1995). Fall applications of *Beauveria bassiana* have been shown to be relatively stable and overwintered with no loss in viability (Gaugler et al. 1989). We found no differences in the concentrations or persistence between fall and spring applications, which suggests *M. anisopliae* does overwinter. This provides flexibility for sugarbeet growers in that they could apply ARS-T1 in the fall or spring, whichever would most easily fit in with individual production practices. The majority of the conidia were isolated from the top 7.5 cm of the soil profile, but there was a trend for more inoculum at greater depths with more years of applications. This trend could be due to more conidial movement over time, increased persistence at greater depths, or a combination of these two factors. To better understand establishment, augmentation, and persistence of *M. anisopliae*, we need critical baseline information such as the concentrations immediately after application and during the 1st year of application.

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