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# Mortalité et infection du ver fil de fer, *Agriotes obscurus* [Coleoptera: Elateridae], par des applications inondatives de *Metarhizium anisopliae* au champ

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Résumé de l'article

Dans une tentative d'infecter mortellement le ver fil de fer *Agriotes obscurus* [Coleoptera: Elateridae], le *Metarhizium anisopliae* a été appliqué au champ selon des combinaisons factorielles d'une formulation granulaire de conidies à 3,68 g de granules ou 1,25 x $10^{10}$ ufc par 196 $cm^2$ (6,38 x $10^7$ conidies $cm^{-2}$), de conidies mélangées à du sol à 1,26 x $10^{10}$ ufc par 2,986 $cm^3$ de sol (4,22 x $10^6$ conidies $cm^{-3}$ de sol) et de graines de blé enrobées de conidies (100 graines de blé ou 4,16 x $10^9$ ufc par 196 $cm^2$ = 2,12 x $10^7$ conidies $cm^{-2}$). Pendant cinq périodes d'échantillonnage, un nombre significativement plus grand de vers fil de fer mycosés a été observé pour les traitements comparativement au témoin. Des différences significatives en termes de mortalité totale des vers fil de fer et de vers fil de fer mycosés au champ sont apparues à toutes les périodes, variant de 15 à 82 j après le traitement. Les traitements ont aussi réduit le nombre de vers fil de fer trouvés dans le cylindre central, ce qui indique qu'ils ont eu un effet répulsif. L'infection latente des vers fil de fer s'est manifestée lorsque des spécimens vivants provenant des traitements au champ sont morts après incubation en laboratoire, en nombres significativement plus élevés que ceux provenant du témoin. Les résultats démontrent que le *M. anisopliae* peut être utilisé au champ et peut infecter et tuer les vers fil de fer, mais seulement à des concentrations excédant 4 x $10^6$ conidies $cm^{-3}$ en utilisant l'isolat, le ver fil de fer, ainsi que les conditions décrites dans la présente étude.

# Mortality and infection of wireworm, *Agriotes obscurus* [Coleoptera: Elateridae], with inundative field applications of *Metarhizium anisopliae*

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In an attempt to cause fatal infection of wireworm *Agriotes obscurus* [Coleoptera: Elateridae], *Metarhizium anisopliae* was applied in the field as factorial combinations of conidia formulated as granules at 3.68 g granules or 1.25 x $10^{10}$ cfu per 196 $cm^2$ (6.38 x $10^7$ conidia $cm^{-2}$), as conidia mixed with soil at 1.26 x $10^{10}$ cfu per 2.986 $cm^3$ soil (4.22 x $10^6$ conidia $cm^{-3}$ soil), and as conidia-coated wheat seed (100 wheat seeds or 4.16 x $10^9$ cfu per 196 $cm^2$ = 2.12 x $10^7$ conidia $cm^{-2}$). The treatments resulted in a significantly greater number of mycosed wireworms compared with the control over and during five sampling periods. Significant differences in total wireworm mortality and mycosed wireworms in the field occurred at any time ranging from 15 to 82 d following treatment. The treatments also caused a reduction in the number of wireworms found in the cores, implying that they had a repellent effect. Latent infection of wireworms became apparent after living wireworms from the field treatments died following incubation under laboratory conditions, in numbers significantly greater than the control. This study showed that *M. anisopliae* can be applied in the field and infect and kill wireworms, but only at concentrations exceeding 4 x $10^6$ conidia $cm^{-3}$ with the subject isolate, wireworm species, and field conditions used in this study.

Keywords: *Agriotes*, biocontrol, biopesticide, Elateridae, *Metarhizium*, microbial control, mycoinsecticide, wireworm.

**[Mortalité et infection du ver fil de fer, *Agriotes obscurus* [Coleoptera: Elateridae], par des applications inondatives de *Metarhizium anisopliae* au champ]**

Dans une tentative d'infecter mortellement le ver fil de fer *Agriotes obscurus* [Coleoptera: Elateridae], le *Metarhizium anisopliae* a été appliqué au champ selon des combinaisons factorielles d'une formulation granulaire de conidies à 3,68 g de granules ou 1,25 x $10^{10}$ ufc par 196 $cm^2$ (6,38 x $10^7$ conidies $cm^{-2}$), de conidies mélangées à du sol à 1,26 x $10^{10}$ ufc par 2,986 $cm^3$ de sol (4,22 x $10^6$ conidies $cm^{-3}$ de sol) et de graines de blé enrobées de conidies (100 graines de blé ou 4,16 x $10^9$ ufc par 196 $cm^2$ = 2,12 x $10^7$ conidies $cm^{-2}$). Pendant cinq périodes d'échantillonnage, un nombre significativement plus grand de vers fil de fer mycosés a été observé pour les traitements comparativement au témoin. Des différences significatives en termes de mortalité totale des vers fil de fer et de vers fil de fer mycosés au champ sont apparues à toutes les périodes, variant de 15 à 82 j après le traitement. Les traitements ont aussi réduit le nombre de vers fil de fer trouvés dans le cylindre central, ce qui indique qu'ils ont eu un effet répulsif. L'infection latente des vers fil de fer s'est manifestée lorsque des spécimens vivants provenant des traitements au champ sont morts après incubation en laboratoire, en nombres significativement plus élevés que ceux provenant du témoin. Les résultats démontrent que le *M. anisopliae* peut être utilisé au champ et peut infecter et tuer les vers fil de fer, mais seulement à des concentrations excédant 4 x $10^6$ conidies $cm^{-3}$ en utilisant l'isolat, le ver fil de fer, ainsi que les conditions décrites dans la présente étude.

Mots clés : *Agriotes*, biopesticide, Elateridae, lutte biologique, lutte microbienne, *Metarhizium*, mycoinsecticide, ver fil de fer.

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## INTRODUCTION

Of all the subterranean arthropods, wireworms [Coleoptera: Elateridae] are probably the most widespread and serious agricultural pests worldwide. In a survey of crop profiles for the United States, wireworms were particularly identified as a problem for corn, potato and sugar beet (see http://pestdata.ncsu.edu/cropprofiles/cropprofiles.cfm), and their importance in potato production is underscored by Parker and Howard (2001). Researchers have noted natural infections of *Metarhizium anisopliae* Metchnikoff in wireworms from as early as 1908. Gorham (1923) described the occurrence of *Pennicillium anisopliae* (Metchnikoff) Vuillemin on *Agriotes mancus* (Say) (later disputed as being *Agriotes obscurus* L. by Fox (1961)) from Nova Scotia, Canada. Rockwood (1950) reported the occurrence of *Metarrhizium brunneum* Petch from *Limonius* sp. in the Pacific Northwest, USA, and *M. anisopliae* from *L. californicus* (Mann.) in Idaho, and carried out crude infection experiments to compare the pathogenicity of the two species. Fox and Jaques (1958) reported that *M. anisopliae* was common in a population of *A. obscurus* in Nova Scotia over several years. They carried out simple controlled laboratory studies to determine its value for wireworm management, which were followed by inundative applications to a hay field resulting in the unsuccessful control of *A. obscurus* and *A. sputator* (L.). Fox (1961) documented the occurrence of *M. anisopliae*-infected larvae, pupae and adults in a natural epizootic in Nova Scotia.

Tinline and Zacharuk (1960) carried out experiments with laboratory-derived isolates of *M. anisopliae*. They obtained high mortality of *Hypolithus bicolor* (Esch.) and *Ctenicera aeripennis destructor* (Brown) and recognized the greater virulence obtained compared with previous studies. They further reported the pathogenicity of *M. anisopliae* and *Beauveria bassiana* (Balsamo) Vuillemin to wireworms by screening isolates collected from stock collections of *H. bicolor* and *C. a. destructor*. They tested the isolates on *H. abbreviatus* (Say), *C. aeripennis* (Kirby), and *L. californicus*, and measured the mortality of eggs, larvae and adults in addition to the effect of larvae size and the resulting feeding behaviour. Because of their positive results from a pest control perspective, they concluded that field trials using *M. anisopliae* were warranted (Zacharuk and Tinline 1968), but further reports have not been apparent. Filipchuk *et al.* (1995) carried out field trials in tobacco using the product Metarrhizine (also known as Metarizin (Ivashchenko and Dolgushina 2002; Novosibirsk – the Russian manufacturer)) in Russia and reported very good efficacy that was comparable to the insecticide turbufos (trade name Counter) against *A. tauricus* Heyden, but they did not report the occurrence of infected cadavers.

With the exception of research in Russia (Filipchuk *et al.* 1995) and a few incidental mentions of attempts at field applications of *M. anisopliae* for wireworm control (e.g. Fox and Jaques 1958), wireworm biological control through inundative applications of any biocontrol agent is almost entirely unexplored or unreported. In a report of the current status of insecticidal controls of wireworms in potatoes, Kuhar *et al.* (2003) reported that very little research had been done in this field, and that although there may be potential for using entomopathogenic fungi such as *B. bassiana* or *M. anisopliae*, there is no substantial data on wireworms to date. Since then, Kabaluk *et al.* (2005) reported good efficacy when selected *M. anisopliae* isolates were tested against *C. pruinina* (Horn) larvae and both larvae and adults of *A. obscurus* and *A. lineatus* (L.) in the laboratory, with field applications alluding to a reduction in wireworm (*A. obscurus*) feeding damage to potato tubers. In this paper, we report data showing the induction of mycosis in wireworm *A. obscurus* in response to inundative applications of *M. anisopliae* conidia in the field. We believe this to be the first report of such an occurrence.

## MATERIALS AND METHODS

The *M. anisopliae* isolate used in this study was acquired in 1999 as mycelia emerging from an infected *A. obscurus* larva cadaver found near the town site of Agassiz, British Columbia, Canada (approximately -121.764° W, 49.239° N). While initially identified as *M. anisopliae* using morphological characteristics, it was later characterized as being within a local geographic isolate clad using amplified fragment length polymorphisms (data not shown). The isolate was mass produced using solid state fermentation with sterile rice as the substrate by Bio-Care Technology Pty. Ltd. (Somersby, New South Wales, Australia). The granular formulation used in this experiment consisted of broken rice grains coated with conidia directly from the solid state fermentation process. Some of the granules from the fermentation process were screened to acquire free conidia for the remaining two treatments (described below).

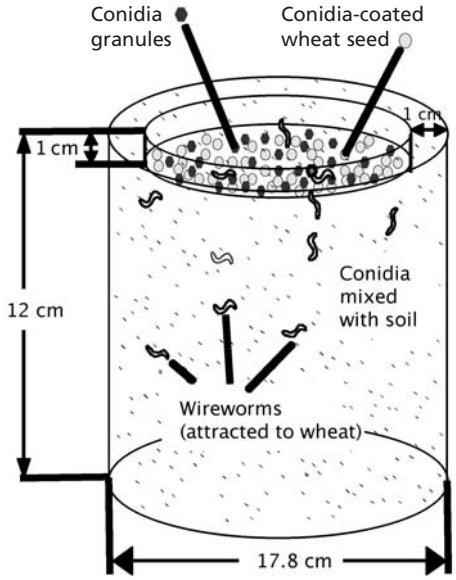


**Figure 1. The experimental unit, a core of soil in the field shown with +levels of each factor, representing treatment 1, for illustrative purposes.**

In the field trial, the experimental unit was a 17.8 cm diam x 12 cm deep (2,986 $cm^3$) core of soil in the field with one of eight combinations of two levels (+ or -) of three *M. anisopliae* treatments: conidia of *M. anisopliae* formulated as granules (abbreviated GRAN; 3.68 g granules or 1.25 x $10^{10}$ colony forming units (cfu) per 196 $cm^2$ or 6.38 x $10^7$ conidia $cm^{-2}$), conidia mixed with soil (abbreviated SOIL; 1.26 x $10^{10}$ cfu per 2.986 $cm^3$ soil or 4.22 x $10^6$ conidia $cm^{-3}$ soil), and conidia-coated wheat seed (abbreviated SEED; 100 wheat seeds or 4.16 x $10^9$ cfu per 196 $cm^2$ or 2.12 x $10^7$ conidia $cm^{-2}$). Thus, the untreated control was soil with minus (-) levels of all three treatments and it contained only wheat seed as a wireworm attractant (Fig. 1). The experiment was arranged in a randomized complete block design with four blocks. Each block was a straight line of soil cores spaced 0.5 m. Each block was divided into five subsample areas with a group of the eight treatments randomized within each subsample area. A single guard core was located at each end of the block. There were five lines of 42 cores within each block (1 m between lines); one line for each of five extraction dates (Fig. 2).

To treat wheat seed with conidia (+SEED), canola oil was used to adhere conidia to the surface of the seed by placing 3 kg of wheat seed in the stainless steel mixing bowl of a Hobart industrial mixer. While the seed was stirred with a whisk attachment, 24 mL canola oil was slowly added to create a light coat of oil evenly over each seed. After stirring, half of the oil-coated seed was removed from the bowl to serve as the –SEED treatment. To coat seed with conidia, 60.9 g of conidia was slowly added to the remaining oil-coated wheat seed while it was stirred for 10 min at medium speed. To mix conidia with soil (+SOIL), 400 cores of soil (20 cores /block*harvest date) were extracted from the field trial site using a golf course cup cutter with the coring barrel reconstructed to 17.8 cm diam. After the soil cores were bulked and placed in a large industrial feed mixer, 156 g of conidia were added to the soil and the two were mixed thoroughly. For soil not receiving conidia (-SOIL), cores were extracted and then kneaded and mixed by hand at the site to simulate the same handling as the soil in the feed mixer. Core cavities were filled with the correspondingly treated soil to approximately 1 cm below the surface soil. A 15.8 cm diam ring was temporarily centred at the height of the core and used to centre 100 wheat seeds spread evenly within the ring for each of the +SEED and –SEED levels. Depending on the treatment (+GRAN or –GRAN), 3.68 g of conidia granules were spread evenly within the ring. After the SEED and GRAN treatments were applied, the soil cores were topped up with 1 cm of soil corresponding to their SOIL level (Fig. 1). Wheat seed served as a wireworm attractant (Vernon *et al.* 2003) for all treatments or an intended wireworm control treatment if coated with conidia or if not treated to lure wireworms into the cores where they would encounter the other two treatments.

On each extraction date (extraction date 1: 15 d; extraction date 2: 27 d; extraction date 3: 48 d; extraction date 4: 68 d; and extraction date 5: 82 d after establishment of the treatments), soil cores were removed to a depth of 12 cm with the 17.8 cm diam golf course cup cutter. The number of living, dead asymptomatic (no sign of *M. anisopliae* infection) and mycosed (exhibiting *Metarhizium* infection mortality) wireworms were enumerated in each soil core. The living wireworms were surface sterilized for 3 min using 5% bleach, double rinsed in sterile water and incubated individually under high humidity at 15°C, with a single wheat seed for food. After being incubated for up to 34 d, the number of living, dead asymptomatic, and mycosed wireworms were enumerated.

Exploratory analysis revealed non-normal data distributions due to '0' responses and variance heterogeneity between extraction dates. Some of the

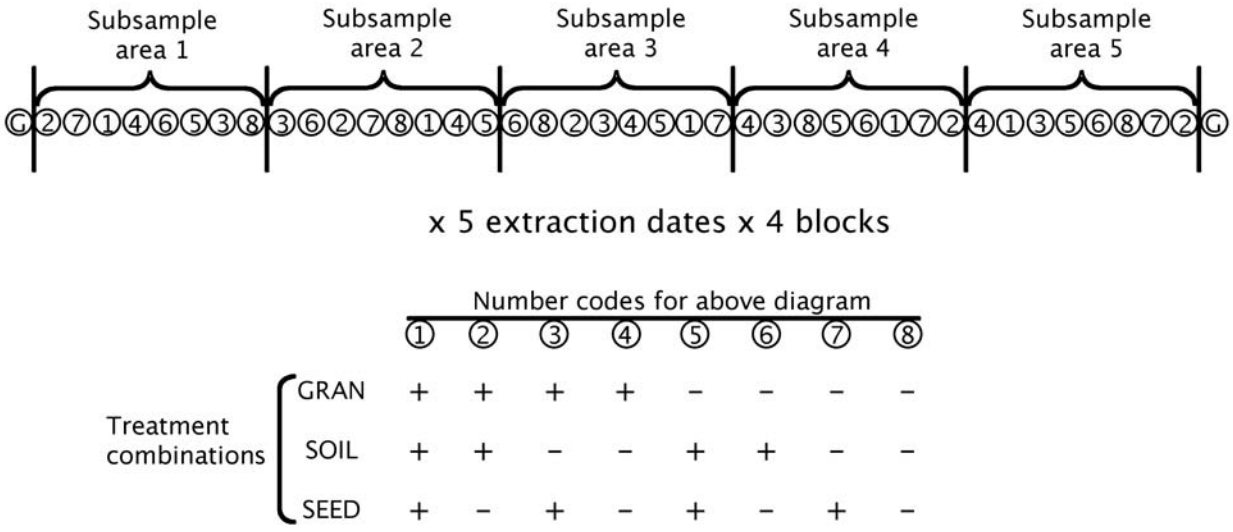


**Figure 2. Layout of field experiment.** Each circle represents a core of soil as shown in Figure 1. GRAN = *Metarhizium anisopliae* conidia formulated as granules, SOIL = conidia mixed with soil, and SEED = conidia-coated wheat seed. G is an untreated guard.

non-normality and variance heterogeneity was alleviated by eliminating those extraction dates where all responses were zero. Data were analyzed with the PROC GLIMMIX procedure of SAS (SAS Institute Inc. 2005), with the effects of treatment and extraction date considered to be fixed. The model was configured with a Poisson/log distribution/link function to account for the skewed right data distribution. The model fit criterion (corrected Akaike's information criterion) revealed that it was not beneficial to model the random variation associated with blocks and subsampling or to model the covariance associated with repeated measurements across extraction dates. The model fit criterion also revealed that it was beneficial to account for variance heterogeneity among extraction dates. Fixed effects were declared significant at $P < 0.10$. The means and standard errors for the analysis of the data were back-transformed using the inverse link function in PROC GLIMMIX.

## RESULTS AND DISCUSSION

Significant differences were found for main effects (*M. anisopliae* treatment and extraction date) for all dependant variables, but no treatment effect was found for mean total wireworm mortality in the field (Table 1). The interaction between treatment and extraction date was significant for mean total wireworm mortality in the field and after incubation, and for mean number of mycosed wireworms after incubation. When contrasts were performed by extraction date, significant treatment differences were found for all dependant variables on various extraction dates. Wireworms were fatally infected in the field in response to inundative applications of *M. anisopliae*. Substantial infection occurred beginning 48 d after the establishment of the treatments in the field (Table 2). However, after living wireworms were incubated and their mortality combined with those from the field, infection in response to the treatments was apparent and significant as early as 27 d after treatment (all treatments except treatment 6 (+SOIL only)). Treatment 6 had no effect on any of the mortality or mycosis variables, despite the soil concentration exceeding that which routinely causes 100% mortality in laboratory bioassays that are conducted using $10^6$ conidia $g^{-1}$ soil. In other studies, we have found that persistence of conidia of the isolate used in this study has been variable in field soil. Most often, cfu have remained relatively unchanged over time for up to 8 wk. In one study, there was an initial decline in cfu, but the level recovered after 2 wk, thus suggesting germination and growth of mycelia in the soil (Kabaluk *et al.* 2007). However, wireworms have been shown to succumb to *M. anisopliae* infection at greater frequency with higher soil moisture (Kabaluk *et al.* 2007), and low soil moisture may have been the reason why treatment 6 had no effect on mortality and mycosis.

It was difficult to discern the effect of dose (the sum of treatment combination doses) and best application method (granules, spores mixed with soil, or treated seed) as mortality trends were not consistent within this context. The significant reduction in mean total wireworm mortality in the field on d 48 for treatment 5 was considered an anomaly and likely related to the fact that fewer wireworms were attracted to the soil cores (see corresponding mean number of wireworms).

The use of the GRAN, SOIL and SEED treatments in this field trial was intended to present different opportunities for wireworms to contract *M. anisopliae* infection rather than to determine which of these treatments would be the best application method. We conclude that inundative applications of *M. anisopliae* caused, and can cause, an increase in field mycosis of wireworms. That living wireworms died and exhibited mycosis after incubation implies that wireworms are able to carry the disease as a latent infection, with full expression (mycelia proliferation, sporulation) occurring under different environmental conditions.

We consider the results somewhat conservative given that the (mean total) number of wireworms found in treated soil cores was most often less than the number found in the control. However, Kabaluk *et al.* (2005) showed that wireworms were

**Table 1. Analysis of variance results (*P* values) for *Metarhizium anisopliae* conidia inundative field application effects on wireworm *Agriotes obscurus* numbers and mortality**

| | Mean number of wireworms per soil core | Mean total wireworm mortality per soil core in the field | Mean number of mycosed wireworms per soil core in the field | Mean total wirewom mortality per soil core after incubation | Mean number of mycosed wireworms per soil core after incubation |
|---|---|---|---|---|---|
| Effect / contrast | | | *P* value | | |
| Extraction date (E) | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** |
| Treatment (T) | **0.049** | 0.516 | **0.018** | **<0.001** | **<0.001** |
| E x T | 0.353 | 0.268 | 0.681 | **<0.001** | **<0.001** |
| 15 d[a] | **0.056** | **0.034** | [b] | 0.122 | [b] |
| 27 d | 0.969 | 0.794 | [b] | **<0.001** | **<0.001** |
| 48 d | 0.197 | 0.133 | 0.145 | **0.042** | **<0.001** |
| 68 d | 0.323 | 0.944 | **0.095** | 0.396 | 0.404 |
| 82 d | 0.300 | 0.195 | 0.811 | 0.111 | 0.361 |

[a] Contrast testing the overall effect of the treatment (T) at each extraction date (E).
[b] Data were very close to 0 and were not included in the analysis.

**Table 2. *Metarhizium anisopliae* conidia inundative field application effects on wireworm *Agriotes obscurus* mean numbers and mortality.** Significant differences from control (Treatment 8) are indicated in bold (***P* < 0.01; **P* < 0.05; no asterisk *P* < 0.1). + treatment applied; - treatment not applied.

| Treatments | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Granules | + | + | + | + | - | - | - | - |
| Soil | + | + | - | - | + | + | - | - |
| Seed | + | - | + | - | + | - | + | - |

| Days after initial treatement | Mean number of wireworms per soil core | | | | | | | | Consolidated standard error |
|---|---|---|---|---|---|---|---|---|---|
| 15 d | 1.75 | **0.85*** | 1.20 | 1.35 | 2.20 | 2.25 | 2.05 | 2.00 | (0.36) |
| 27 d | 9.65 | 10.00 | 10.60 | 9.20 | 9.00 | 8.55 | 8.80 | 9.70 | (1.34) |
| 48 d | **2.35** | 2.95 | 5.00 | 3.40 | 2.85 | 3.80 | 2.90 | 4.05 | (0.70) |
| 68 d | 1.15 | 1.15 | 1.75 | 2.15 | 1.50 | 1.85 | 1.10 | 1.60 | (0.35) |
| 82 d | **0.55** | **0.30*** | **0.65** | **0.65** | **0.55** | **0.65** | **0.65** | 1.40 | (0.29) |
| All | **2.18*** | **1.95**** | 2.70 | 2.53 | **2.43** | 2.71 | **2.32*** | 2.99 | (0.23) |
| | Mean total wireworm mortality per soil core – in the field | | | | | | | | |
| 15 d | **0.20*** | <0.01 | <0.01 | 0.05 | **0.20*** | 0.05 | 0.10 | <0.01 | (0.06) |
| 27 d | <0.01 | <0.01 | 0.05 | 0.05 | 0.05 | <0.01 | 0.10 | 0.10 | (0.06) |
| 48 d | 0.56 | 0.32 | 0.70 | 0.68 | **0.16** | 0.35 | 0.42 | 0.50 | (0.15) |
| 68 d | 0.11 | 0.25 | 0.21 | 0.25 | 0.30 | 0.10 | 0.25 | 0.15 | (0.13) |
| 82 d | 0.05 | <0.01 | **0.15*** | <0.01 | 0.05 | <0.01 | <0.01 | <0.01 | (0.04) |
| All | 0.17 | 0.10 | 0.20 | 0.18 | 0.15 | 0.09 | 0.17 | 0.14 | (0.04) |
| | Mean number of mycosed wireworms per soil core – in the field | | | | | | | | |
| 15 d | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | -- |
| 27 d | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | -- |
| 48 d | 0.38 | 0.26 | **0.45*** | **0.47*** | 0.11 | 0.15 | 0.21 | 0.10 | (0.12) |
| 68 d | 0.11 | **0.25*** | **0.21** | **0.25*** | **0.20** | <0.01 | 0.15 | <0.01 | (0.08) |
| 82 d | 0.05 | <0.01 | 0.15 | <0.01 | 0.05 | <0.01 | <0.01 | <0.01 | (0.07) |
| All | **0.17** | **0.16** | **0.26**** | **0.23**** | 0.12 | 0.05 | 0.12 | 0.03 | (0.05) |
| | Mean total wireworm mortality per soil core – after incubation | | | | | | | | |
| 15 d | **0.20*** | <0.01 | <0.01 | 0.05 | **0.20*** | 0.05 | 0.10 | <0.01 | (0.07) |
| 27 d | **6.37**** | **5.35**** | **5.37**** | **5.63**** | **3.95**** | 1.35 | **3.25**** | 1.45 | (0.58) |
| 48 d | 1.88 | 1.47 | **2.55**** | **2.21*** | 1.16 | 1.10 | 1.74 | 1.00 | (0.39) |
| 68 d | 1.16 | 1.15 | 1.79 | 2.15 | 1.50 | 1.85 | 1.10 | 1.60 | (0.37) |
| 82 d | - | - | - | - | - | - | - | 1.40 | (0.24) |
| All | **1.43*** | 1.13 | **1.53**** | **1.59**** | 1.18 | 0.89 | 1.13 | 0.98 | (0.13) |
| | Mean number of mycosed wireworms per soil core – after incubation | | | | | | | | |
| 15 d | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | -- |
| 27 d | **5.21**** | **4.35**** | **4.11**** | **4.84**** | **2.79**** | 0.65 | **1.80**** | 0.30 | (0.51) |
| 48 d | **1.38**** | **1.21**** | **1.85**** | **1.58**** | 0.74 | 0.70 | **0.89*** | 0.30 | (0.23) |
| 68 d | 0.42 | **0.50** | 0.37 | **0.50** | **0.50** | 0.10 | 0.25 | 0.05 | (0.18) |
| 82 d | 0.05 | 0.15 | 0.30* | 0.10 | 0.15 | 0.05 | 0.10 | <0.01 | (0.09) |
| All | **1.17**** | **1.13**** | **1.26**** | **1.23**** | **0.84**** | 0.34 | **0.64**** | 0.15 | (0.11) |

not deterred from entering a soil-conidia environment up to $10^8$ conidia $g^{-1}$ soil. Although there was some emigration from a soil-conidia environment, it was reduced if wheat was present as a food source. All treatments, including the control, contained wheat seed in this experiment.

Because no treatment differences were found for any mortality or mycosis variable in treatment 6 (+SOIL only), it appears that with the *M. anisopliae* isolate–wireworm species combination used in this study, very high doses (well in excess of $4 \times 10^6$ conidia $cm^{-3}$ soil – the approximate concentration of treatment 6) of conidia are required to infect an appreciable number of wireworms under field conditions, or that individual conidia in soil under field conditions (such as in treatment 6) may be an unsuitable state for them to be infective to wireworms. High concentrations could be accomplished economically if wireworms can be attracted to focussed areas of inoculum. Fortunately, this is attainable as wireworms are chemotropic and attracted to $C0_2$ sources such as germinating wheat seeds (Doane *et al.* 1975). Treatment effects were more pronounced after combining field mortality and mortality of living wireworms incubated after extraction of the cores, indicating that wireworms exposed to the treatments possessed a higher incidence of latent infection of *M. anisopliae* compared with the control. Whether these wireworms would have eventually died of *M. anisopliae* infection in the field is uncertain. However, a few wireworms in the untreated control exhibited mycosis as well, and since carrying out this experiment we have observed many cases where seemingly healthy wireworms collected in the field, surface sterilized and stored in sterile soil died of *M. anisopliae* infection at 15°C, confirming the occurrence of latent infections in nature (personal observation).

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