Spinosad Interacts Synergistically with the Insect Pathogen *Metarhizium anisopliae* Against the Exotic Wireworms *Agriotes lineatus* and *Agriotes obscurus* (Coleoptera: Elateridae)

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ABSTRACT We determined that spinosad interacts synergistically with the biocontrol agent *Metarhizium anisopliae* (Metch) Sorokin to increase the mortality of two wild-collected wireworm species, *Agriotes lineatus* (L.), and *Agriotes obscurus* (L.). Bioassays were performed using a *M. anisopliae* isolate originally acquired from a local wireworm cadaver. *M. anisopliae* was applied as a soil drench at 3.3×10^2 and 10^4 conidia per gram sand, respectively. Soil drenches also were prepared using a commercial formulation of the actinomycete toxins spinosyn-A and spinosyn-D (common name spinosad) at sublethal doses of 1.5, 3, and 6 ppm active ingredient per gram sand. Combined treatments of spinosad and *M. anisopliae* were synergistic in causing mortality for all spinosad concentrations. Wireworm feeding activity was reduced after exposure to both spinosad and *M. anisopliae* and was found to be concentration dependent. The high mortality and reduced rate of wireworm feeding suggest that spinosad and *M. anisopliae* treatment combinations should be tested in the field.

KEY WORDS biological control, interaction, naturalyte, wireworm, Metarhizium

Two exotic species of wireworm *Agriotes lineatus* (L.), and *A. obscurus* (L.) (Coleoptera: Elateridae) are established in British Columbia as significant crop pests, especially of potato, *Solanum tuberosum* L., and corn, *Zea mays* L. (Vernon and Pats 1997, Vernon et al. 2001). The wireworm's threat to agriculture is due to several factors, including their 3–6-yr larval development, their polyphagous diet, their hardiness, and their difficulty to target in the subterranean environment. In Canada, no treatments are available after planting, and most pesticides previously used to control wireworm have been withdrawn from registration. With unchecked expansion of wireworm populations and few crops having registered treatment options, a viable pest management strategy is needed.

Hyphomycetes are commonly encountered insect pathogens and are described as facultative filamentous fungi that reproduce via asexual conidia on solid substrate and via yeast-like hyphal bodies within the host (McCauley and Zacharuk 1968, Goettel and Inglis 1997). *Metarhizium anisopliae* (Metch) Sorokin (Hypocreales: Clavicipitaceae) is a ubiquitous fungus found globally in most soils. It is known to be pathogenic to a wide range of insects (Huxham et al. 1989); this wide host range is facilitated by many unique isolates of *M. anisopliae* that are able to produce a suite of cuticle degrading proteases (St Leger et al. 1987) and immunosuppressive destruxins toward the host insect (Zacharuk 1981). Other differences in observed virulence are due to factors related to the host's health, developmental stage, and its particular ability to resist infection (Gillespie et al. 2000). Soil temperature, moisture, and exposure time to conidia also have been shown to be important contributors in causing mortality. *M. anisopliae* causes wireworm mortality in the laboratory, and inundative field applications have been associated with wireworm mycosis and reduced feeding damage in potato crops (Kabaluk et al. 2005). However, when wireworm populations were high, the sole application of *M. anisopliae* was not sufficient in preventing the potato crop from sustaining economically significant feeding damage.

Similar deficiencies with other control agents have led to the formulation of binary treatments of biological and physical controls with insecticides (Pachamuthu and Kamble 2000, Furlong and Groden 2001), nematodes (Ansari et al. 2004), boric acid (Zurek et al. 2002), diatomaceous earth (Akbar et al. 2004), and other biological agents (Inglis et al. 1997, Thomas et al. 2003) or metabolites (Brousseau et al. 1998) to synergize the total control potential. It is unclear why or how these combined treatments interact, but it is clear that the physiological effects caused by one agent can increase the virulence of the biological agent and thereby cause higher mortality. These studies also indicate that combination formulations use less of each active ingredient than would be required if they were to be applied individually. Although many treat-

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ment combinations interact synergistically, and cause significantly higher mortality than predicted, not all combinations interact favorably. Some combinations are antagonistic and cause lower mortality than predicted; thus, it can be difficult to foresee which products will compliment each other.

Spinosad is registered under a new class of reduced-risk insecticides called naturalytes, compounds that are produced by living organisms. Spinosyns are produced by the actinomycete *Saccharopolyspora spinosa* (Actinomycetales: Pseudonocardiaceae), and are known to be active against many noxious pests as well as some parasitoids and pollinators, but they are otherwise harmless to nontarget organisms (Wilson et al. 2003). Spinosyns are classified as macrolides and are neurotoxic through the inhibition of nicotinal acetylcholine receptors, and GABA receptors by means of an undefined mode of action (Salgado 1998, Wilson et al. 2003).

Here, we report tests of the interaction of *M. anisopliae* and spinosad in causing mortality by exposing the wireworms to different dose combinations. We found that a metabolite from an unrelated soil microbe increases the virulence of an entomopathogenic fungus.

Materials and Methods

Wireworm Collection and Selection. Wireworms were collected from land with a 30-yr history of pesticide-free management (UBC Farm, Vancouver, British Columbia, Canada). The species composition, determined through pheromone trap catches of adult males was found to consist of two dominant species, *A. lineatus* and *A. obscurus*, with an estimated prevalence of 88 and 12%, respectively. After collection, the mixed population of wireworms was transferred to 27-liter plastic tubs, kept at 7°C in the soil, and wireworms were periodically fed wheat, *Triticum aestivum* L., seed. Twenty-four hours before the bioassay, the tubs of soil were warmed to 22°C, and 8 h before the bioassay, sections of potato were buried near the soil surface to attract actively feeding wireworm. From these wireworms, those within a 10-cm range of the potato bait, and within a 19–24-mm range in length, were selected for experimentation. The masses of wireworms ranged from 32 to 42 mg, and the size range corresponded to *A. lineatus* instars 11–13 (Kabanov 1975). *A. lineatus* and *A. obscurus* are difficult to distinguish by their larval characteristics alone (Wilkinson 1963), and because both species are pests to local crops, no attempt was made to identify each wireworm beyond the genus.

Preliminary Bioassay. Industrial sand (Target Products Ltd., Burnaby, British Columbia, Canada), and course sands of varying particle size (0.1–0.4 mm in diameter) were rinsed 10 times, heat sterilized, and equivalent amounts of each were thoroughly mixed. To each 35-ml plastic cup, 30 g of the sand mixture was added and subsequently hydrated to 7% moisture (wt:wt). Spinosad and conidia treatments were both applied as a drench (described below), and the volume of fluid added was included in the total moisture calculation such that each plastic cup received equivalent hydration.

Three stock solutions of spinosad (Tracer, DOW Agrosciences, Indianapolis, IN) were prepared that were 0, 45, and 90 ppm (AI)/ml. One milliliter of each stock spinosad solution was added to each plastic cup to raise the sand concentration to 0, 1.5, and 3 ppm (AI)/g. Two levels of *M. anisopliae* treatments (as described below) were applied as a 1-ml drench (0 and 1.42×10^6 conidia per ml). The *M. anisopliae* isolate was acquired from a local, naturally infected *Agriotes* sp. cadaver, and was grown in pure culture on 10-cm petri plates of Sabaroud dextrose agar amended with yeast (SDAY) culture media at 28°C for 18 d. Conidia were washed with 25 ml of distilled H_2O (dH_2O) amended with 0.0025% Triton X-100, and the stock suspension was kept in total darkness at room temperature until application the same day. Germination of the conidia was determined 20 h after spreading on the SDAY media.

Both spinosad and *M. anisopliae* treatments were mixed into the sand with a glass rod, and sterile tap water was added to adjust the moisture to 7% (wt:wt). Each wireworm was weighed to verify uniformity of size and was then placed individually in a 35-ml plastic cup filled with the sand mixture. One *T. aestivum* wheat seed was pushed into the sand of each plastic cup so that it was just below the surface, and then fitted, unventilated lids were applied to the plastic cups. Five replicate cups from each treatment combination were randomized in a covered plastic tray lined with moistened paper towels to maintain humidity. Three replicate trays were prepared, covered with aluminum foil to maintain darkness, and kept at a constant 22°C for duration of trial. Mortality was assessed periodically throughout the trial by mechanically stimulating the wireworm with a blunt object. Wireworm were considered dead when they no longer moved their mandibles or prolegs after stimulation.

Synergy Bioassay. The preliminary assay indicated that additional concentrations of conidia and spinosad should be tested. Thus, the methods described above were followed exactly, except four levels of spinosad (0, 1.5, 3, and 6 ppm/g sand) were combined with three levels of conidia (0, 3.3×10^2, and 3.3×10^4 conidia per g sand). Four stock solutions of spinosad were prepared at 0, 45, 90, and 180 ppm (AI)/ml, and 1 ml of each stock solution was added to each cup to raise the sand concentration to 0, 1.5, 3, and 6 ppm (AI)/g. Conidia prepared from the same isolate described above were washed from the petri plate with 25 ml of dH_2O amended with 0.025% Triton X-100, and the stock suspension was adjusted to a concentration of 10^4 and 10^6 conidia per ml with an Improved Neubauer hemocytometer. The resulting suspension was kept in total darkness at room temperature until application the same day. Mortality was determined as described above. The trial was repeated twice.

Feeding Activity. On each evaluation date, the amount of feeding that had occurred on each wheat seed and the proportion of wireworms feeding in each

Table 1. Preliminary bioassay: wireworm mortality from spinosad and *M. anisopliae* treatment combinations on each sampling date 10–60 d after exposure

Treatment		Time (d)	Wireworm mortality (%)[a]				χ^{2b}
Fungus (spores/g)	Spinosad (ppm)		Fungi	Spinosad	Expected	Observed	
10^5	1.5	10	13.3	0	13.3	0	13.3*
		20	20.0	0	20	13.3	2.24
		30	26.7	0	26.7	33.3	1.63
		40	33.3	0	33.3	66.7	33.5*
		50	33.3	0	33.3	73.3	48.1*
		60	46.7	0	46.7	80.0	23.7*
10^5	3	10	13.3	0	13.3	0	13.3*
		20	20.0	0	20	13.3	2.24
		30	26.7	0	26.7	60.0	41.5*
		40	33.3	0	33.3	73.3	48.1*
		50	33.3	0	33.3	86.7	85.6*
		60	46.7	0	46.7	93.3	46.5*

[a] Average mortality from 15 wireworm per treatment combination.
[b] A chi-square comparison that exceeds 3.84, with df = 1 and $\alpha = 0.05$, is considered synergistic and is denoted by an asterisk (*).

bioassay tray were recorded. If the wheat seed had been consumed, another seed was added to the plastic cup, and if the wireworm had died, its feeding activity was not included in the analysis.

Fungus Viability. For each experiment, conidia viability was determined by enumerating the percentage of germinated conidia 20 h after spreading on fresh SDAY media. One hundred microliters of a conidia suspension ($\approx 10^7$ conidia per ml, 0.025% Triton X-100) was spread on a 110-mm petri plate containing 20 ml of SDAY media (30 g/liter SDAY agar; Sigma-Aldrich, St. Louis, MO). Plates were then incubated at 27°C, in complete darkness for 20 h. Germination percentage was determined by first placing three 18-mm square coverslips directly on media surface and then by counting the number of germinated conidia and total number of conidia per field of view at 250× magnification. Three to four fields of view were observed per coverslip, so that a minimum of 10 counts was performed for each petri plate. Conidia were considered germinated when a distinct germ tube had formed.

Combination Treatment Compatibility. Because studies have shown that insecticides can interfere with growth and sporulation of *M. anisopliae* (Li and Holdom 1994), it was important to determine whether spinosad was fungicidal against *M. anisopliae.* Quantitative microplate assays of filamentous fungal growth have identified a linear relationship between the change in biomass and the change in absorbance at particular wavelengths (Broekaert et al. 1990). These methods are sensitive to fungal growth, and they were used to identify fungicidal effects of spinosad, and the concentrations that should be avoided during coapplication.

To each well of the 96-well, polystyrene, flat-bottomed microplate (Sigma-Aldrich), 100 μl of Saboraud's dextrose broth amended with yeast extract (15 g/liter SDAY, Sigma-Aldrich), 10 μl from one of 11 concentrations of spinosad, and 10 μl from a conidia suspension (0 and 200 spores per μl) was added. Final spinosad concentrations of 768, 384, 192, 96, 48, 24, 12, 6, 3, 1.5, and 0 ppm AI (vol:vol) per well were tested with and without 2,000 spores of *M. anisopliae* per well for changes in biomass. All cultures were incubated at 25°C under continuous darkness and were shaken at 150 rpm until spectrometer analysis. Absorbance was measured at 595 nm every 24 h in an automated plate-reader (SpectraMax 190, Molecular Devices, Sunnyvale, CA) up to 96 h after starting the experiment. The absorbance from each sample containing only spinosad was subtracted from the corresponding absorbance of the spinosad and fungus combinations, so that differences in absorbance were only due to increases in biomass of the fungus.

Experimental Design and Statistical Analysis. For the preliminary assay, two levels (0 and 10^6 conidia per g sand) of *M. anisopliae* and three levels (0, 1.5, and 3) of spinosad were analyzed as a complete random design. Fifteen replicates of each treatment combination were prepared, and 10 repeated measurements of the percentage of mortality were taken over the 60-d trial. Expected mortality (E) was generated from the following formula: $E = O_{Spin} + O_{Met}(1 - O_{Spin})$, where E is the expected mortality, and O_{Spin} and O_{Met} represent the proportion mortality due to treatments of pure spinosad and pure *M. anisopliae,* respectively. This formula has been used in similar studies to evaluate the effects of combined treatments (Trisyono and Whalon 1999, Hummelbrunner and Isman 2001). The predicted effects of spinosad and *M. anisopliae* treatments (E) were compared with the observed mortality of the binary treatments (O) with the following formula, $\chi^2 = \{(O - E)^2\}/E$.

For the synergy bioassay, each of the 12 treatment combinations, three levels (0, 10^2, and 10^4 conidia per g sand) of *M. anisopliae* and four levels (0, 1.5, 3, and 6 ppm [AI]) spinosad were replicated 15 times per trial with 10 repeated measurements of proportion mortality, and feeding status over 60 d. A Kaplan–Meier survivorship analysis was performed to determine whether the addition of spinosad had an effect on the wireworm's time to death. For synergy effects, the expected mortality (E) was generated by the formula described above and was compared with the observed mortality (O) of the binary treatments by a

Table 2. Mean survival time of *Agriotes* sp. wireworms after exposure to each treatment combination of *M. anisopliae* and spinosad

Spinosad concn (ppm)	Survival time (d)[a]		
	0 *M. anisopliae* (conidia/g)	10^2 *M. anisopliae* (conidia/g)	10^4 *M. anisopliae* (conidia/g)
0	61.78 (2.79)	59.38 (3.07)	32.75 (3.13)
1	58.78 (2.96)	45.8 (3.38)	27.78 (2.39)
3	58.3 (3.05)	46.53 (3.44)	23.53 (2.45)
6	52.28 (3.54)	47.63 (3.31)	22.33 (1.53)
Wilcoxon test	$\chi^2 = 5.56^b$	$\chi^2 = 11.64^b$	$\chi^2 = 9.71^b$
	$P = 0.1352$	$P = 0.0087$	$P = 0.0212$

[a] Standard error shown in parentheses.
[b] Wilcoxon tests performed for each level of fungus.

chi-square assessment. All statistical analysis was performed with JMP IN version 5.1 statistical software package (SAS Institute 2005).

For the feeding activity assay, the percentage of individuals feeding at each sampling date was averaged over two experiments, and the treatment means were subject to analysis of variance (ANOVA).

For the compatibility assay, the growth rate was determined by the slope of the regression line from the absorbance data. Four replicate wells were pre-

Table 3. Synergy bioassay: wireworm mortality from spinosad and *M. anisopliae* treatment combinations on each sampling date 15–60 d after exposure

Treatment		Time (d)	Wireworm mortality (%)[a]				
Fungus (spores/g)	Spinosad (ppm)		Fungi	Spinosad	Expected	Observed	χ^{2b}
10^2	1.5	15	0	5 (5)	5.0	2.5	1.25
		20	0	5 (5)	5.0	7.5	1.25
		27	0	5 (5)	5.0	15.0	20.00*
		34	2.5 (2.5)	5 (5)	7.4	15.0	7.88*
		40	6.3 (3.8)	5 (5)	11.0	23.8	14.95*
		50	7.5 (2.5)	15.5 (5)	21.4	37.5	12.16*
		60	28.5 (11.3)	20 (10)	43.0	45	0.09
10^2	3	15	0	5 (5)	5.0	15.0	20.00*
		20	0	5 (5)	5.0	22.5	61.25*
		27	0	10 (5)	10.0	25.0	22.50*
		34	2.5 (2.5)	12.5 (7)	14.7	35.7	30.06*
		40	6.3 (3.8)	16.2 (14)	21.5	51.3	41.40*
		50	7.5 (2.5)	22.5 (17)	28.3	60	35.46*
		60	28.5 (11.3)	22.5 (17)	44.8	78.8	25.76*
10^2	6	15	0	5 (5)	5.0	15	20.00*
		20	0	5 (5)	5.0	27.5	101.25*
		27	0	5 (5)	5.0	27.0	96.80*
		34	2.5 (2.5)	7.5 (5)	9.8	30	41.53*
		40	6.3 (3.8)	13.8 (6.3)	19.2	42.5	28.16*
		50	7.5 (2.5)	15 (5)	21.4	57.5	61.05*
		60	28.5 (11.3)	18.8 (1.3)	42.2	65.0	12.34*
10^4	1.5	15	22.5 (7.5)	5 (5)	26.4	37.5	4.69*
		20	35.5 (5.0)	5 (5)	38.3	57.5	9.69*
		27	55 (15)	5 (5)	57.3	65	1.05
		34	70 (5.1)	5 (5)	71.5	75	0.17
		40	72.5 (7.5)	5 (5)	73.9	86.3	2.09
		50	75 (5)	15 (5)	78.8	95	3.35
		60	83.8 (3.8)	20 (10)	87.0	93.8	0.53
10^4	3	15	22.5 (7.5)	5 (5)	26.4	42.5	9.86*
		20	35.0 (5)	5 (5)	38.3	70.0	26.35*
		27	55.0 (15)	10 (5)	59.5	85.0	10.93*
		34	70.0 (5.1)	12.5 (7)	73.8	90.0	3.58
		40	72.5 (7.5)	16.2 (14)	77.0	92.5	3.14
		50	75.0 (5)	22.5 (17)	80.6	95.0	2.56
		60	83.8 (3.8)	22.5 (17)	87.4	97.5	1.16
10^4	6	15	22.5 (7.5)	5.0 (5)	26.4	52.5	25.88*
		20	35.5 (5)	5.0 (5)	38.3	72.5	30.67*
		27	55.0 (15)	5.0 (5)	57.3	80.0	9.04*
		34	70.0 (5.1)	7.5 (5)	72.3	85.0	2.25
		40	72.5 (7.5)	13.8 (6.3)	76.3	98.7	6.58*
		50	75.0 (5)	15.0 (5)	78.8	100	5.73*
		60	83.8 (3.8)	18.8 (10)	86.8	100	1.99

[a] Average mortality from 30 wireworm; standard deviation is in parentheses.
[b] Chi-square comparison that exceeds 3.84, with df = 1 and $\alpha = 0.05$, is considered synergistic and is denoted by an asterisk (*).

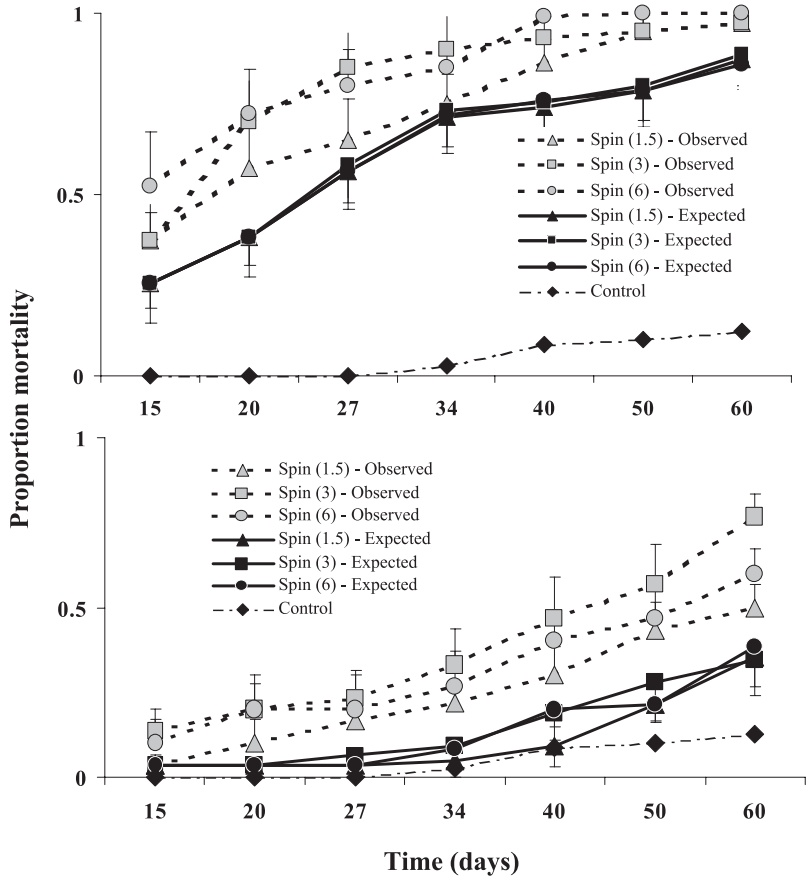

Fig. 1. Observed and expected effects of spinosad on wireworm mortality at two concentrations of *M. anisopliae.* (Top) High rate of conidia. Combination treatments consist of spinosad at 0 (diamond), 1.5 (triangle), 3 (square), and 6 (circle) ppm soil concentration with *M. anisopliae* at 3.3×10^4 conidia/g sand. (Bottom) Low rate of conidia. Combination treatments consist of spinosad at 0 (diamond), 1.5 (triangle), 3 (square), and 6 (circle) ppm soil concentration with *M. anisopliae* at 3.3×10^2 conidia/g sand. The average percentage of mortality is reported along with the standard error.

pared for each treatment combination, two replicate microplates were prepared for each trial, and each trial was repeated twice. Four repeated measurements of absorbance were taken at 24, 48, 72, and 96 h after the initiation of experiment. The slopes from all trials for each spinosad level were subject to ANOVA, and means were separated via the Tukey's honestly significant difference (HSD) test ($\alpha = 0.05$).

Results

Preliminary Bioassay. Thirty days after exposure, combined treatments of spinosad and *M. anisopliae* produced significantly higher mortality than expected at spinosad concentrations of 1.5 and 3 ppm (Table 1). The synergistic results led to the design of a more sensitive factorial experiment with additional levels of spinosad and fungus. No wireworm mortality was observed in any of the spinosad-only or control treatments, which indicated that the levels tested were sublethal.

Synergy Bioassay. The Kaplan–Meier survivorship analysis detected significant differences in the time to death due to spinosad for both levels of fungus (Table 2). *M. anisopliae* levels of 10^2 and 10^4 conidia per g soil were deemed low to medium based on data from previous trials (Kabaluk et al. 2005). However, when a locally obtained isolate was applied as a drench, conidia at 10^2/g sand caused 29% mortality over the 60-d trial, and conidia at 10^4/g caused 84% mortality after 60 d. Spinosad treatments caused symptoms consistent with neurological poisoning and only caused a low level of mortality when applied alone. When spinosad and *M. anisopliae* were applied together as a combination treatment, mortality was significantly higher than the expected value of their additive effect, which indicates a synergistic interaction. Based on individual treatment levels, the greatest synergistic effect occurred when 10^2 conidia per g sand were used with 6 ppm spinosad (Table 3). Similar mortality was achieved at this dose as that in the levels of fungi 2 orders of magnitude higher without spinosad (Fig. 1). In the 10^4 conidia per g treatments, mortality was consistent in all treatments, but it was higher than predicted when spinosad was added.

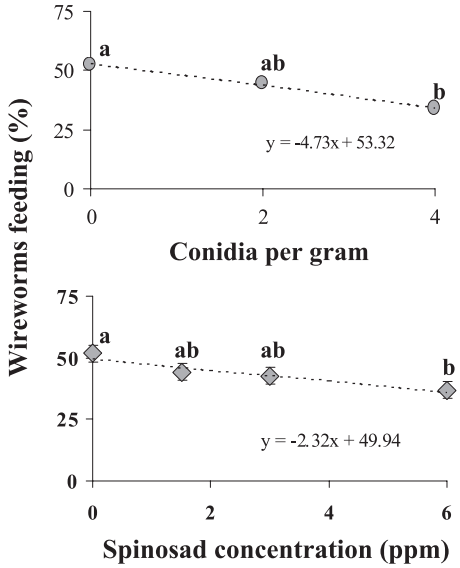

Fig. 2. Average percentage of surviving wireworm feeding while under continuous exposure to treatment combinations. (Top) *M. anisopliae* was found to cause significant reductions in feeding, that are concentration dependent ($F = 12.163$; df = 2, 93; $P < 0.0001$). (Bottom) Spinosad treatments also were found to cause significant concentration-dependent reductions in feeding ($F = 3.3639$; df = 3, 92; $P = 0.0220$). Means followed by the same letter are not significantly different (Tukey's HSD; $\alpha = 0.05$).

Feeding Activity. Over 60 d, wireworm behavior alternated between periods of intense feeding and periods of inactivity. Sometimes, larvae fed only on the embryos of the germinated seed, and other times they consumed the entire seed and all shoot and root growth. The average percentage of wireworm feeding activity decreased as both *M. anisopliae* concentration and spinosad concentration increased (Fig. 2).

Fungus Viability. Conidia viability was determined for each bioassay as well as for each compatibility assay to verify the quality of spores. In all experiments, *M. anisopliae* conidia germination was found to be 96% or higher after 20 h on SDAY media.

Combination Treatment Compatibility. The concentration of spinosad caused *M. anisopliae* to grow at significantly different rates than the control ($F = 13.28$; df = 10, 22; $P < 0.0001$). Although a nonsignificant increase in growth rate was detected at low concentrations of spinosad, the mean growth rate was significantly decreased when spinosad concentrations were 192 ppm or higher (Fig. 3). This result indicates that spinosad and *M. anisopliae* are compatible in the soil at the rates tested in the bioassay, but if both conidia and spinosad are to be applied as a drench they should not be applied in the same solution when spinosad concentrations exceed 96 ppm.

Discussion

In this study, we found that a combined treatment of spinosad and *M. anisopliae* caused significantly

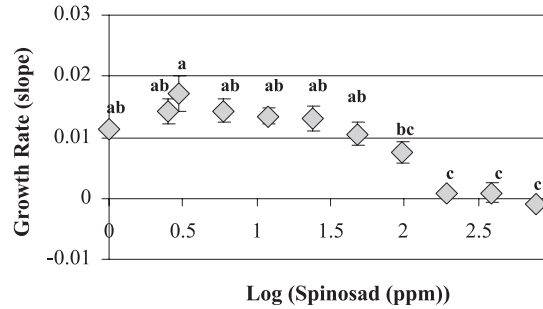

Fig. 3. Average *M. anisopliae* growth rate during continuous exposure to spinosad concentrations ranging from 0 to 768 ppm active ingredient (vol:vol). Means followed by the same letter are not significantly different (Tukey's HSD; $\alpha = 0.05$).

higher mortality than either treatment alone. This suggests that low levels of a reduced-risk pesticide can be combined with a biological agent to reduce wireworm populations without traditional pesticide strategies.

The mechanism for the observed interaction between spinosad and *M. anisopliae* remains unclear. However, given that *M. anisopliae* produces toxins that interrupt the metabolism and immune function of many insects (Suzuki et al. 1971, Zacharuk 1981), these molecules may have to be detoxified by the insect to prolong its survival. As a result, when a wireworm becomes infected, several resistance pathways may be induced, including cellular or humeral immune mechanisms to destroy the pathogen as well as detoxifying pathways to clear the metabolites. Because *M. anisopliae* proliferates as yeast-like hyphal fragments in the host's hemocoel (Goettel and Inglis 1997), it is reasonable to expect toxin levels in the hemolymph to increase as the number of fungal cells increases. If death of an insect is dependent on the type and rate of toxins produced by the pathogen, then a compound that inhibits any of the detoxifying mechanisms would increase the speed of kill. Despite that spinosyns are neurotoxins that act by way of a novel mechanism, they also may alter the sensitivity of the wireworm to fungal toxins or the potency of its immune response to *M. anisopliae.*

A positive side effect of the treatments is that both spinosad and *M. anisopliae* reduce larval feeding, even though the average percentage of individuals feeding was never <50% of the control group. Although intermittent feeding behavior is typical of *Agriotes* wireworms (Furlan 1998), it is also known that both immune responses (Rolff and Siva-Jothy 2003) and detoxification processes (Ahmad et al. 1987) are biochemically expensive to sustain. Therefore, growing wireworms may rely on nutritional inputs to facilitate their response against *M. anisopliae* for detoxification of the xenobiotic molecules, or both, and thus must feed.

Wireworm need several years to develop and many infested fields consist of larval populations of mixed ages, stages of development (Kabanov 1975), and spe-

cies (Brian 1947, Vernon and Pats 1997). Thus, a viable biological control treatment must be able to overcome abiotic factors as well as immunological and behavioral differences associated with the mixed population. Because *M. anisopliae* is known to gradually degrade in the soil, the inclusion of spinosad treatments could extend the total control period provided by one application of the fungus. This laboratory study suggests that field trials should be carried out to determine whether combined treatments of spinosad and *M. anisopliae* are practical and effective against exotic wireworms.

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References Cited

Ahmad, S., L. B. Brattsten, C. A. Mullin, and S. J. Yu. 1987. Enzymes involved in the metabolism of plant allelochemicals, pp. 74–129. *In* L. B. Brattstein and Sami Ahmad [eds.], Molecular aspects of insect-plant associations. Kluwer Academic Publishers, Amsterdam, The Netherlands.

Akbar, W., J. C. Lord, J. R. Nechols, and R. W. Howard. 2004. Diatomaceous earth increases the efficacy of *Beauvaria bassiana* against *Tribolium castaneum* larvae and increases conidia attachment. J. Econ. Entomol. 97: 273–280.

Ansari, M. A., L. Tirry, and M. Moens. 2004. Interaction between *Metarhizium anisopliae* CLO 53 and entomopathogenic nematodes for the control of *Hoplia philanthus.* Biol. Control 31: 172–180.

Brian, M. V. 1947. On the ecology of beetles of the genus *Agriotes* with special reference to *A. obscurus.* J. Anim. Ecol. 16: 210–224.

Broekaert, W. F., F. R. G. Terras, P. A. Bruno, C. Vanderleyden, and J. Vanderleyden. 1990. An automated quantitative assay for fungal growth inhibition. Microbiol. Lett. 69: 55–60.

Brousseau, C., G. Charpentier, and S. Belloncik. 1998. Effects of *Bacillus thuringiensis* and destruxins (*Metarhizium anisopliae* mycotoxins) combinations on spruce budworm (Lepidoptera: Tortricidae). J. Invertebr. Pathol. 72: 262–268.

Furlan, L. 1998. The biology of *Agriotes ustulatus* Schaller (Col., Elateridae). II. Larval development, pupation, whole cycle description and practical implications. J. Appl. Entomol. 122: 71–78.

Furlong, M. J., and E. Groden. 2001. Evaluation of synergistic interactions between the Colorado potato beetle (Coleoptera: Chrysomelidae) pathogen *Beauvaria bassiana* and the insecticides, imidicloprid, and cyromazine. J. Econ. Entomol. 94: 344–356.

Gillespie, J. P., A. M. Bailey, B. Cobb, and A. Vilcinskas. 2000. Fungi as elicitors of insect immune responses. Arch. Insect Biochem. Physiol. 44: 49–68.

Goettel, M. S., and G. D Inglis. 1997. Chapter V-3 Fungi: hyphomycetes, pp 409. *In* L. Lacey [ed.], Manual of techniques in insect pathology. Academic, San Diego, CA.

Huxham I. M., K. D. Z. Samuels, J. B. Heale, and N. J. McCorkindale. 1989. In vivo and *in vitro* assays for pathogenicity of wild-type and mutant strains of *Metarhizium anisopliae* for three insect species. J. Invertebr. Pathol. 53: 143–151.

Hummelbrunner, L. A., and M. B. Isman. 2001. Acute, sublethal, antifeedant, and synergistic effects of monoterpenoid essential oil compounds on the tobacco cutworm, *Spodoptera litura* (Lep., Noctuidae). J. Agric. Food Chem. 49: 715–720.

Inglis, G. D., D. L. Johnson, K. J. Cheng, and M. S. Goettel. 1997. Use of pathogen combinations to overcome the constraints of temperature on entomopathogenic hyphomycetes against grasshoppers. Biol. Control 8: 143–152.

Kabaluk, J. T., M. Goettel, M. Erlandson, J. Ericsson, G. Duke, and R. Vernon. 2005. *Metarhizium anisopliae* as a biological control for wireworms and a report of some other naturally-occurring parasites. IOBC/WPRS Bull. 28: 109–115.

Kabanov, V. A. 1975. Uber vorkommen und entwicklung von *Agriotes lineatus* (Coleoptera, Elateridae) im europaischen Teil der USSR. Pedobiologia Bd. 15S: 98–105.

Li, D. P., and D. G. Holdom. 1994. Effects of pesticides on growth and sporulation of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes). J. Invertebr. Pathol. 63: 209–211.

McCauley, V. J. E., and R. Y. Zacharuk. 1968. Histopathology of green muscardine in larvae of four species of Elateridae (Coleoptera). J. Invertebr. Pathol. 12: 444–459.

Pachamuthu, P., and S. Kamble. 2000. In vivo study on combined toxicity of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) strain ESC-1 with sublethal doses of chlorpyrifos, propetamphos, and cyfluthrin against German cockroach (Dictyoptera: Blattellidae). J. Econ. Entomol. 93: 60–70.

Rolff, J., and M. T. Siva-Jothy. 2003. Invertebrate ecological immunology. Science (Wash., D.C.) 301: 472–475.

SAS Institute. 2005. Jump start statistics – a guide to statistics and data analysis using JMP and JMP IN software, 3rd ed., version 5.1.3. Thompson Brooks/Cole, Belmont, CA.

Salgado, V. L. 1998. Studies on the mode of action of spinosad: insect symptoms and physiological correlates. Pestic. Biochem. Physiol. 60: 91–102.

St. Leger, R. J., A. K. Charnley, and R. M. Cooper. 1987. Characterization of cuticle-degrading proteases produced by the entomopathogen *Metarhizium anisopliae.* Arch. Biochem. Biophys. 253: 221–232.

Suzuki, A., K. Kawakami, and S. Tamura. 1971. Detection of destruxins in silkworm larvae infected with *Metarhizium anisopliae.* Agric. Biol. Chem. 35: 1641–1643.

Thomas, M. B., E. L. Watson, and P. Valverde-Garcia. 2003. Mixed infections and insect-pathogen interactions. Ecol. Lett. 6: 183–188.

Trisyono, A., and M. E. Whalon. 1999. Toxicity of neem applied alone and in combinations with *Bacillus thuringiensis* to Colorado potato beetle (Coleoptera: Chrysomelidae). J. Econ. Entomol. 92: 1281–1288.

Vernon, B., and P. Pats. 1997. Distribution of two European wireworms, *Agriotes lineatus and A. obscurus* in British Columbia. J. Entomol. Soc. Br. Columbia 94: 59–61.

Vernon, R. S., E. Lagasa, and H. Philip. 2001. Geographic and temporal distribution of *Agriotes obscurus* and *A. lineatus* (Coleoptera: Elateridae) in British Columbia and Washington as determined by pheromone trap surveys. J. Entomol. Soc. Br. Columbia 98: 257–265.

Wilson, T., J. Valle, and E. Viñuela. 2003. Is the naturally derived insecticide Spinosad® compatible with insect natural enemies? Biocontrol Sci. Technol. 13: 459–475.

Wilkinson, A. T. S. 1963. Wireworms of cultivated land in British Columbia. Proc. Entomol. Soc. Br. Columbia 60: 3–17.

Zacharuk, R. Y. 1981. Chapter 13: fungal diseases of terrestrial insects, pp 367. *In* W. Davidson [ed.], Pathogenesis of invertebrate microbial diseases. Allanheld, Osmun Publishers, Totowa, NJ.

Zurek, L., D. W. Watson, and C. Schal. 2002. Synergism between *Metarhizium anisopliae* (Deuteromycota: Hyphomycete) and boric acid against the German cockroach (Dictyoptera: Blattellidae). Biol. Control 23: 296–302.

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