RESEARCH/INVESTIGACIÓN

EFFECT OF DINOTEFURAN, INDOXACARB, AND IMIDACLOPRID ON SURVIVAL AND FITNESS OF TWO ARIZONA-NATIVE ENTOMOPATHOGENIC NEMATODES AGAINST *HELICOVERPA ZEA* (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

Navarro, P. D., J. G. McMullen II, and S. P. Stock. 2014. Effect of dinotefuran, indoxacarb, and imidacloprid on survival and fitness of two Arizona-native entomopathogenic nematodes against *Helicoverpa zea* (Lepidoptera: Noctuidae). Nematropica 44:64-73.

The effect of three insecticides commonly used in Arizona, dinotefuran, indoxacarb, and imidacloprid, was evaluated on two Arizona-native entmopathogenic nematodes (EPN), *Heterorhabditis sonorensis* (Caborca strain) and *Steinernema riobrave* (SR-5 strain), using *Helicoverpa zea* (Lepidoptera: Noctuidae) as the insect host. Specifically, we assessed their effect on EPN survival and fitness (virulence and reproduction). Three application timings were considered: i) EPN applied first, insecticide applied 24 h later, ii) insecticide applied first, EPN applied 24 h later, and iii) simultaneous application of EPN and insecticide. Our results showed that infective juvenile (IJ) survival of *S. riobrave* and *H. sonorensis* was not significantly affected by the application of the selected insecticides. Indoxacarb had an ambiguous effect on the *S. riobrave* life cycle showing a synergistic effect in the virulence of this nematode but reducing its progeny production by two-fold. Similar results were observed for nematode progeny production when *H. sonorensis* and indoxacarb were applied simultaneously. All combinations of imidacloprid were antagonistic to the virulence of *S. riobrave* but additive with respect to the virulence of *H. sonorensis*. Dinotefuran had an additive effect in all combinations and timings evaluated for both EPN species. The negative effect of indoxacarb in the progeny of the tested EPN species suggests this insecticide may have an impact in the recycling of IJs in the soil.

Key words: Heterorhabditis sonorensis, interactions, *Steinernema riobrave*, synthetic insecticides.

RESUMEN

Navarro, P. D., J. G. McMullen II, and S. P. Stock. 2014. Efecto de dinotefuran, indoxacarb e imidacloprid en la supervivencia y eficacia de dos especies de nematodos entomopatógenos nativos de Arizona sobre *Helicoverpa zea* (Lepidoptera: Noctuidae). Nematropica 44:64-73.

En esta investigación se evaluó el efecto de tres insecticidas comúnmente usados en Arizona, dinotefuran, indoxacarb e imidacloprid con dos especies de nematodos entomopatogenos (NEP) nativos de Arizona: *Heterorhabditis sonorensis* (cepa Caborca) y *Steinernema riobrave* (cepa SR-5). Específicamente, se evaluó su efecto en la supervivencia, virulencia y reproducción, de ambos NEP. Tres tiempos de aplicación fueron considerados: i) insecticida aplicado 24 h antes del NEP, ii) insecticida y NEP aplicados simultáneamente, iii) insecticida aplicado 24 después del NEP. Los resultados mostraron que la supervivencia de los infectivos juveniles de *S. riobrave* y *H. sonorensis* no fue afectada significativamente por la aplicación de los insecticidas seleccionados. Indoxacarb tuvo un efecto ambiguo en el ciclo de vida de *S. riobrave*. Por ejemplo, las aplicaciones de indoxacarb después o simultáneamente a *S. riobrave* tuvieron un efecto sinérgico en la virulencia de este nematodo. Sin embargo, la producción de progenie fue reducida a la mitad. De manera similar, la aplicación simultanea de *H. sonorensis* e indoxacarb redujo la progenie de este nematodo a la mitad. Todas las combinaciones de imidacloprid resultaron antagonistas en la virulencia de *H. sonorensis*. Dinotefuran tuvo un efecto aditivo en todas las combinaciones evaluadas por ambas especies de NEP. El efecto negativo de indoxacarb en la progenie de los NEP evaluados sugiere que este insecticida puede tener un impacto en el reciclaje de IJs en el suelo.

Palabras clave: Heterorhabditis sonorensis, insecticidas sintéticos, interacciones, *Steinernema riobrave*.

INTRODUCTION

In Arizona, insecticides such as neonicotinoid and oxadiazine are widely used for the control of lepidopterous pests of lettuce, melons, and other crops (Barkley and Ellsworth, 2004;; Prabhaker *et al*., 2005; Palumbo and Castle, 2009; Kerns and Palumbo, 2009). However, the excessive use of these products has generated serious problems including selection for insecticide resistance (Nauen and Denholm, 2005), outbreaks of secondary pests (Szczepaniec *et al*., 2011), and environmental concerns (Lacey *et al*., 2001). In this respect, environmentally friendly alternatives such as microbial entomopathogens and entomopathogenic nematodes (EPN) including Steinernematidae and Heterorhabditidae have been suggested to ameliorate the negative consequences of these and other chemical pesticides (Zimmermann, 1993; Tadeusz *et al*., 1998; McCoy *et al*., 2002; Shapiro-Ilan *et al.*, 2006a; Ansari *et al*., 2007; Ebssa and Koppenhöfer, 2012). The rationale of combining natural pathogens and synthetic insecticides is to achieve better control of a single pest through a synergistic or additive effect on the mortality of the targeted pest (Barbosa *et al*., 1996; Koppenhöfer and Grewal, 2005). Moreover, it is expected that the amount of pesticide used would be less than the amount considered for the application of this pesticide alone. In addition to this positive outcome, it is anticipated that resistance and outbreak of secondary pests would also be diminished (Lacey *et al*., 2001).

Entomopathogenic nematodes have been shown to be a suitable non-chemical alternative for the control of a wide range of insect pests. Indeed, many investigations have shown their efficacy when applied alone or combined with other entomopathogens and (or) with synthetic insecticides (Zimmerman and Cranshaw, 1990; Koppenhöfer and Kaya, 1998; Koppenhöfer *et al*., 2000, 2002; Grewal *et al*., 2004; Barbara and Buss, 2005; Shapiro-Ilan *et al*., 2009, 2012).

Many studies have evaluated the compatibility of EPN with chemical insecticides and assessed two key components: i) survival of the nematodes in tank mix, and ii) the effect of the nematode-insecticide combination on the targeted pest (Rovesti *et al*., 1988; Vainio and Hokkanen, 1990; Koppenhöfer and Grewal, 2005; Shapiro-Ilan *et al*., 2006b;). In this study, we evaluated the effect of three commonly used insecticides (imidacloprid, indoxacarb, and dinotefuran) on the survival, virulence, and reproduction of two Arizona-native EPN species, *Heterorhabditis sonorensis* (Caborca strain) and *Steinernema riobrave* (SR-5 strain). The corn earworm, *Helicoverpa zea* (Lepidoptera: Noctuidae), an important insect pest of vegetable crops in southwestern USA was the insect host for all experiments.

MATERIALS AND METHODS

Insects, Nematodes, and Insecticides

The corn earworm, *H. zea* (Lepidoptera: Noctuidae), was considered as the insect host for all experiments. This insect was selected because it is a major pest in various crops including leaf vegetables, sweet corn, and melons in Arizona and other southwestern states (Palumbo and Castle 2009). Eggs of *H. zea* were obtained from Benzon Research (Carlside, PA) and reared under laboratory conditions at 28°C and 80% relative humidity following procedures described by Waldbauer *et al*. (1984). Fourth instar larvae were used in all assays. This larval stage was chosen because it is the stage that moves from the feeding site (aerial part of the plant) to the soil for pupation; and in this environment, it can encounter EPN. During experiments, larvae were fed with 5 g of corn earworm artificial diet (Southland Products Inc., Lake Village, AR).

Two Arizona-native EPN species, *S. riobrave* and *H. sonorensis*, were studied. Nematodes were propagated in vivo using the fifth instar of *Galleria mellonella* (Lepidoptera: Pyrallidae) following procedures described by Kaya and Stock (1997). Infective juveniles less than 10 d old (the time since initial emergence from insect cadavers) were used in each assay and stored at 15°C. Three synthetic insecticides, dinotefuran [ScorpionTM 35SL] (Gowan, Yuma, AZ), indoxacarb [Avaunt®] (Dupont, Wilmington, DE), and imidacloprid [Merit® 75 WP] (Bayer, NC) were evaluated.

Insecticide and EPN Dose-Response Assays

Different concentrations of insecticides were evaluated to determine their lethal concentration 50 (LC_{50}) on *H. zea* and the two EPN species considered in this study. Insecticide concentrations were selected as follows: i) imidacloprid and dinotefuran: 1, 2, 4, 8, 12 g (ai)/ha and ii) indoxacarb: 1, 2.5, 25, 250, and 2,500 g (ai)/ha.

Assays to determine insecticide LC_{50} on *H. zea* were conducted in SOLO® plastic cups (1 oz; 10-cm diam.). Each cup was filled with 4 g of sterile sand (Quikrete® Play Sand, fine mesh) and one *H. zea* larva was placed individually in each cup. Larvae were fed once with 5 g of artificial diet at the beginning of the experiment. Positive controls consisted of 1 ml of nematode inoculum and each insecticide alone. Negative controls consisted of the application of 1 ml of distilled water per cup. There were 10 cups for each treatment. Treatments were organized in blocks, and each block of 10 cups was repeated three times. Insect mortality was recorded daily for 10 d after initial inoculation.

Different EPN doses were also considered to determine their LC_{50} toward *H. zea* larvae. Evaluated doses were: 1, 5, 10, 100, 200 IJ/ml for each EPN species. Doses were calculated based on preliminary assays conducted by Navarro (unpubl. data). For these experiments, 1 ml of nematode suspension was evenly dispensed on the sand surface for each cup. Treatments (different EPN concentrations) were organized in blocks where each block consisted of 10 cups. Each block was repeated three times. Insect mortality was recorded daily for 10 consecutive days after initial inoculation.

Insecticide-EPN Compatibility Assays

Twelve-well plates (Corning® Costar® with 22-mm-diam. wells) were considered as the experimental arena for the insecticide-EPN compatibility experiments. Each EPN species was evaluated separately. One ml of each insecticide/concentration considered in the above experiments was mixed with 100 IJs and added into each well. There were six replicates for each insecticide type/insecticide concentration/EPN species combination. Each experiment was repeated three times. The number of dead nematodes was recorded 10 d after initial setup. Nematodes were examined under a dissecting microscope (30×) and were probed with a needle to check for motility. Nematodes that responded to the probing were considered alive and unresponsive nematodes were considered dead.

Effect of Insecticides on EPN Virulence and Reproductive Fitness Assays

EPN virulence was measured by considering percentage of insect mortality and IJ establishment (the number of nematodes that successfully invaded each insect). Entomopathogenic nematodes' reproductive fitness was measured as the total number of IJs that emerged from each cadaver in a 10-d period after initial emergence. The effect of different application timings of the selected insecticides on each EPN species was also evaluated. Three applications were used: i) EPN applied first, insecticide applied 24 h later, ii) insecticide applied first, EPN applied 24 h later, and iii) one simultaneous application of EPN and insecticide. Each nematode species was evaluated separately.

EPN Virulence and IJ Establishment

Rates of nematode (*S. riobrave*: 3 IJ/ml; *H. sonorensis*: 7 IJ/ml) and insecticide (obtained from the dose-response assays) were used to assess EPN virulence and IJ establishment. The assays were conducted in SOLO® cups filled with 4 g of sterile sand, where a single *H. zea* larva was added to each cup. Each cup received 1 ml of inoculum containing nematodes and (or) insecticide, which was applied at different times. Positive controls consisted of 1 ml of nematode inoculum and each insecticide alone. Negative controls consisted of the application of 1 ml of distilled water per cup. Ten larvae (1 larva = 1 replicate) were evaluated for each treatment and controls. The experiment was arranged in a completely randomized block design. Each block was conducted three times. Cups were incubated at 25 ± 1°C and 80% RH. Larval mortality was recorded daily during the 10 d post-inoculation. Cadavers with typical coloration of EPN infection were removed from the cups. Half of the cadavers (15 in total) were dissected to record IJ establishment (after 72 h) using the enzymatic digestion method described by Mauleón *et al*. (1993). The remaining 15 cadavers were used to record progeny production.

EPN Reproductive Fitness

Cadavers were thoroughly rinsed in distilled water and individually placed in modified White traps (Kaya and Stock, 1997). Daily observations were made to record the first day of IJ progeny emergence. Emerging IJs were collected from each White trap for 10 d after the first day of emergence, as described by Koppenhöfer and Kaya (1998), and stored in 250-ml tissue culture flasks at 4°C until counted. Insect cadavers that did not produce progeny were not included.

Statistical Analysis

All experiments were analyzed by ANOVA using the statistical software JMP® 8.0.2 (SAS Institute, 2008). Tukey's test was considered when differences among means were statistically significant. Nematode mortality data were subjected to probit analyses (Finney, 1964) using the statistical software SPSS (SPSS 20.0, 2012). For experiments where the insect mortality was calculated, the number of dead larvae was recorded, and the percentage mortality was corrected using Abbott's formula (Abbott, 1925). Before analysis, and to meet the assumption of normality, data were arcsine (insect mortality) or $\log_{10}$ (establishment and progeny production) transformed. Insect mortality, nematode establishment, and nematode progeny production were considered as response variables. Timings of application were considered as explanatory variables.

The nature of the interactions (additive, antagonistic, or synergistic) between EPN and insecticides was determined based on the analysis used by Nishimatsu and Jackson (1998). The expected mortality of larvae was calculated based on the formula $P_E = P_o + (1\text{-}P_o)\ (P_1) + (1\text{-}P_o)\ (1\text{-}P_1)\ (P_2)$, where P_E is the expected mortality on combination of EPN and insecticide, P_o is the mortality in the control, P_1 is the mortality after treatment with the insecticide alone, and P_2 is the mortality after treatment with

the nematode alone. The determination of X^2 was calculated through the formula: $X^2 = (L_0 - L_E)2/L_E + (D_0 - D_E)2/D_E$, where L_0 is the number of living larvae observed, L_E is the number of living larvae expected, D_0 is the number of dead larvae observed, and D_E is the number of dead larvae expected. The parameter X^2 was used to test the hypothesis of independence (df = 1 and P = 0.05). Combinations of nematode and insecticide, where $X^2 < 3.84$, were defined as additive. Synergism was denoted by $X^2 > 3.84$ and $P_C > P_E$. Antagonism was defined as $X^2 > 3.84$ and $P_C < P_E$, where P_C is the observed mortality of the insecticide and nematode combination.

RESULTS

Insecticide and EPN Dose Response Assays

The LC_{50} (Slope ± SE with the 95% CL) obtained for the selected insecticides against *H. zea* were the following: imidacloprid 1.4 µ ai/ha^{-1} (1.88 ± 0.5 with 0.4-2.4 CL), dinotefuran 1.0 µ ai/ha^{-1} (3 ± 0.4 with 0.3-1.2 CL), and indoxacarb of 33 µ ai/ha^{-1} (2.1 ± 0.9 with 22-56 CL). When these concentrations of insecticides were tested on *H. sonorensis* and *S. riobrave*, no significant effect on IJ survival was observed ($F_{2,54}$ = 0,12; P = 0.3 for *S. riobrave* and $F_{2,54}$ = 1.2; P = 0.8 for *H. sonorensis*). For example, survival of *H. sonorensis* IJs (average ± SD) in combination with dinotefuran was 95% ± 0.9, with indoxacarb was 92% ± 1.2, and with imidacloprid was 87% ± 2.5. Survival of *S. riobrave* (average ± SD) was 94.5% ± 0.6, with indoxacarb was 86.6 ± 2.3, and with imidacloprid was 91.5 ± 0.4.

The LC_{50} of *H. sonorensis* and *S. riobrave* against *H. zea* was 3 IJs/larva (2.1 ± 0.8 with 2.3 ± 9.1 CL) and 7 IJs/larva (1.6 ± 0.4 with 0.9 – 4.2 CL), respectively. These concentrations were considered appropriate to measure interactions between EPN and insecticides in *H. zea* and were used in subsequent experiments.

Effect of Insecticides on EPN Virulence and Reproductive Fitness

The combined application of dinotefuran (all application timings) and *H. sonorensis* significantly increased the virulence of this nematode (Fig. 1A). A 30% increment on *H. zea* mortality was achieved with any of the tested combinations compared with the application of either nematode or dinotefuran alone. Combinations of *H. sonorensis* with indoxacarb did not show significant differences compared to the application of *H. sonorensis* alone; however, simultaneous application of *H. sonorensis* and indoxacarb resulted in *H. zea* mortality that was significantly higher than with the application of indoxacarb alone (Fig. 1B). Two of the imidacloprid application timings (before or simultaneous), significantly increased mortality of *H. zea*, when compared with the application of *H. sonorensis* or imidacloprid alone (Fig. 1C).

With respect to *S. riobrave*, all combination timings with dinotefuran increased the virulence of this EPN species when compared with the application of *S. riobrave* or dinotefuran alone (Fig. 2A). Applications of indoxacarb after or simultaneously with *S. riobrave* increased the virulence of *S. riobrave* by increasing mortality of *H. zea* when compared with the application of either the nematode or the insecticide alone (Fig. 2B). Application of indoxacarb before *S. riobrave* was statistically similar to the application of nematodes alone. All application timings evaluated involving indoxacarb and *S. riobrave* resulted in higher mortality of *H. zea* than the application of indoxacarb alone.

None of the imidacloprid application timings tested improved the virulence of *S. riobrave* (Fig. 1C). Insect mortality was similar to that observed for the applications of imidacloprid or EPN alone.

Most of the nematode and insecticide combinations and application timings were considered additive (Table 1). Only the two combination of *S. riobrave* with indoxacarb (insecticide applied after or simultaneously with the nematode) had a synergistic effect on *H. zea* mortality. In contrast, antagonistic effects were observed when indoxacarb was applied before *H. sonorensis* and for all applications of imidacloprid with *S. riobrave* (Table 1).

With respect to IJ establishment, none of the insecticides ($F_{2,96}$ = 0,11; P = 0.18 for *H. sonorensis*; $F_{2,96}$ = 0,09; P = 0.9 for *S. riobrave*), and application timings ($F_{3,96}$ = 0,21; P = 0.88 for *H. sonorensis*; $F_{3,96}$ = 0,78; P = 0.5 for *S. riobrave*), or their interactions (insecticide × application type) ($F_{6,96}$ = 0,39; P = 0.87 for *H. sonorensis*; $F_{6,96}$ = 1.04; P = 0.4 for *S. riobrave*), affected the ability of either EPN species to penetrate the insect host. For both nematodes, the average number of penetrating IJ was 1.5 IJs/larva, which was similar to the average number observed in the controls.

EPN Reproductive Fitness

Progeny production of *H. sonorensis* was not significantly affected by the application of dinotefuran and imidacloprid at any of application timings evaluated, ($F_{3,32}$ = 1.3; P = 0.61) (Figs. 3A, 3B, and 3C). In contrast, a 50% reduction in progeny production was observed for the simultaneous application of indoxacarb and this nematode species when compared with the control (Fig. 3B). For both alternate application timings of indoxacarb and *H. sonorensis*, no significant differences on the number of emerging IJs were observed when compared with the control.

For *S. riobrave*, progeny production increased with the application of dinotefuran for all application timings (Fig.4A). However, IJ production was reduced with the application of indoxicarb for all application timings (Fig. 3B). Emerging IJ populations

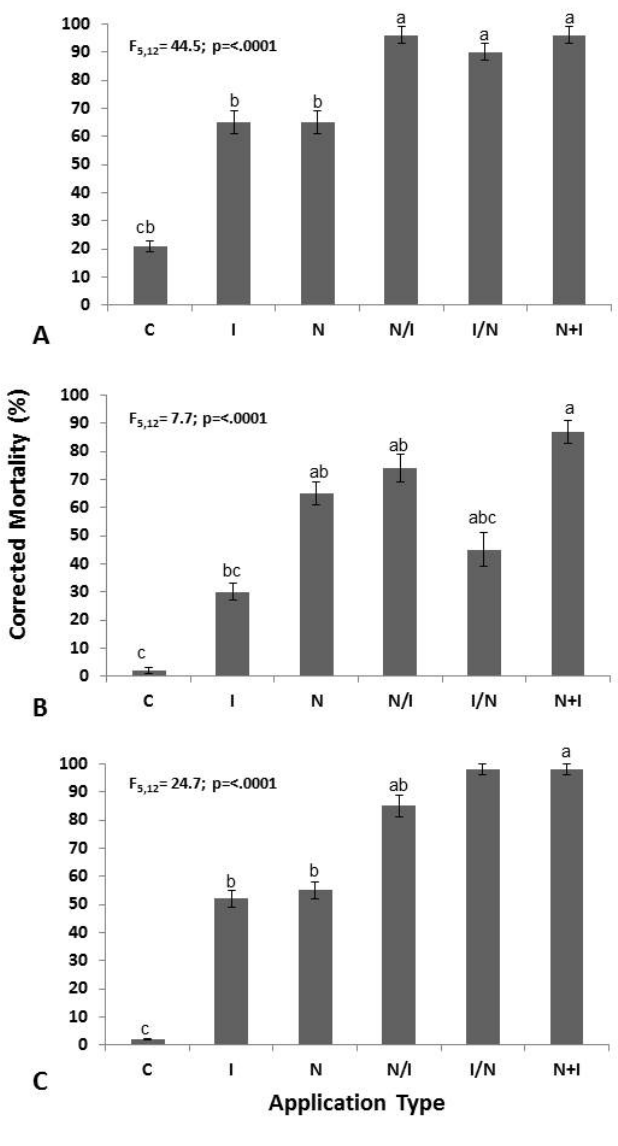

Fig. 1. Corrected mortality (± SE) for *Helicoverpa zea* exposed to combinations of *Heterorhabdotis sonorensis* with different insecticides: A) dinotefuran, B) indoxacarb, C) imidacloprid. Different letters above bars indicate statistical differences (based on Tukey's test, P = 0.05). Data plotted are untransformed. References: C = control (distilled water), I = insecticide, N = nematode, I/N = insecticide first, nematode 24 h later, N/I = nematode first, insecticide 24 h later, N+I = nematode and insecticide applied simultaneously.

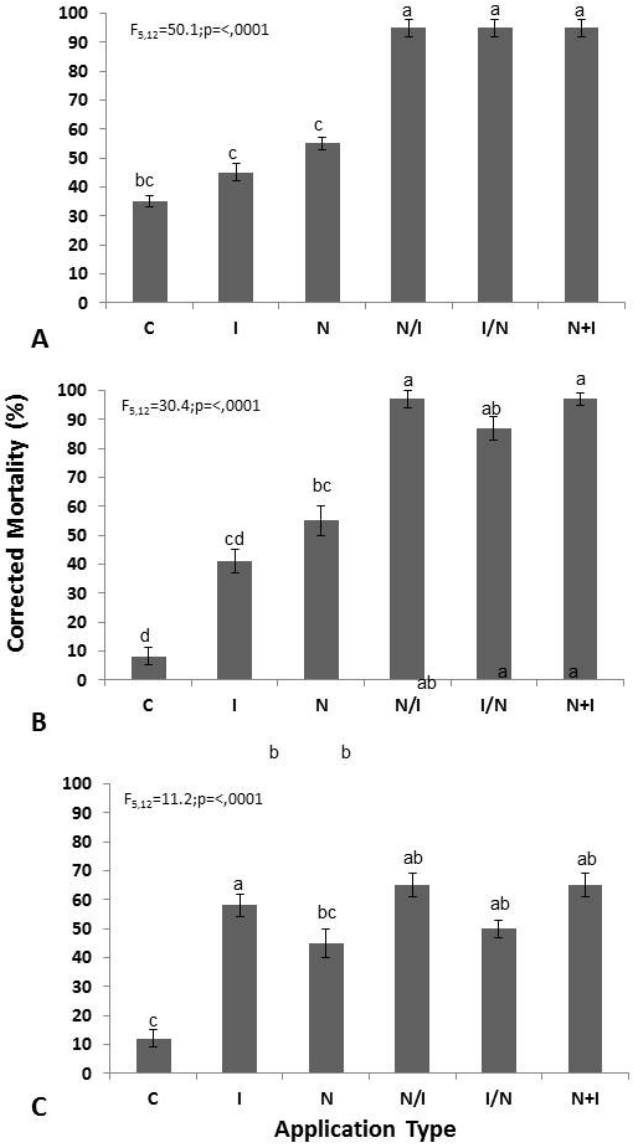

Fig. 2. Corrected mortality (± SE) for *Helicoverpa zea* exposed to combinations of *Steinernema riobrave* with different insecticides: A) dinotefuran, B) indoxacarb, C) imidacloprid. Different letters above bars indicate statistical differences (based on Tukey test, P = 0.05). Data plotted are untransformed. References: C = control (distilled water), I = insecticide, N = nematode, I/N = insecticide first, nematode 24 h later, N/I = nematode first, insecticide 24 h later, N+I = nematode and insecticide applied simultaneously.

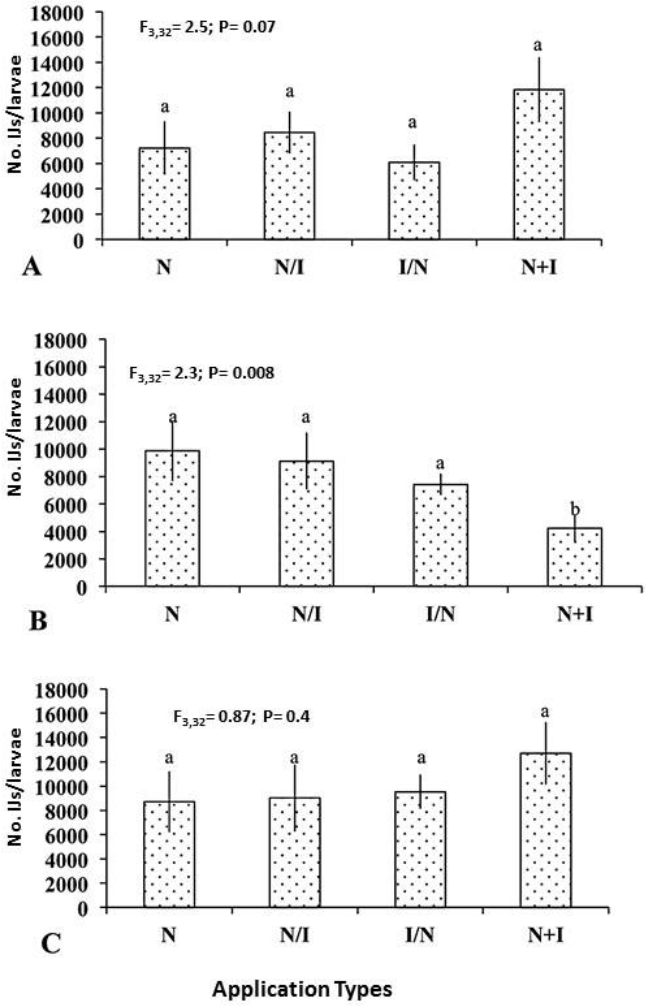

Fig. 3. *Heterorhabditis sonorensis* progeny production (± SE) when applied with: A) dinotefuran, B) indoxacarb, and C) imidacloprid in alternating sequence [insecticide before nematode (I/N) or nematode before insecticide (N/I)] or in mixture (N+I). Different letters above bars indicate statistical differences (based on Tukey's test, P = 0.05). Data plotted are untransformed. References: C = control (distilled water), I = insecticide, N = nematode, I/N = insecticide first, nematode 24 h later, N/I = nematode first, insecticide 24 h later, N+I = nematode and insecticide applied simultaneously.

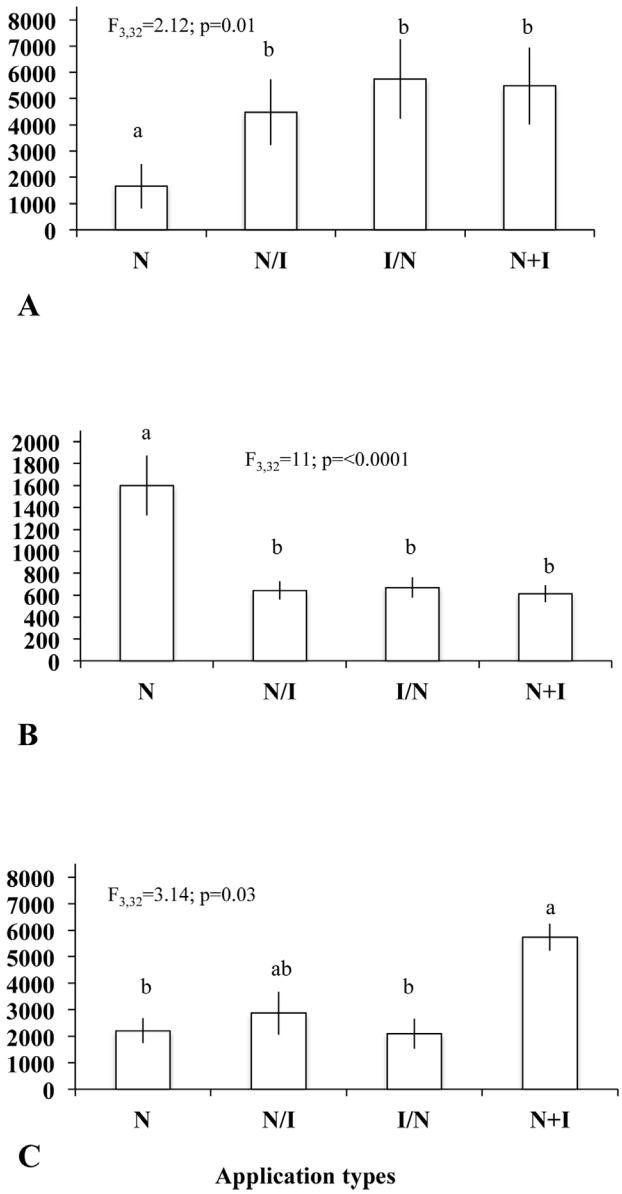

Fig. 4. *Steinernema riobrave* progeny production (± SE) when applied with: A) dinotefuran, B) indoxacarb, and C) imidacloprid in alternating sequence [insecticide before nematode (I/N) or nematode before insecticide (N/I)] or in mixture (N+I). Different letters above bars indicate statistical differences (based on Tukey's test, P = 0.05). Data plotted are untransformed. References: C = control (distilled water), I = insecticide, N = nematode, I/N = insecticide first, nematode 24 h later, N/I = nematode first, insecticide 24 h later, N+I = nematode and insecticide applied simultaneously.

Table 1. Nature of the interactions of entomopathogenic nematodes - insecticide application timings on larval mortality of *Helicoverpa zea*.

		Mortality of *H. zea*							
		H. sonorensis				*S. riobrave*			
Insecticide	Treatment[x]	Obs	Exp	X^2[y]	Interaction[z]	Obs	Exp	X^2[y]	Interaction[z]
Dinotefuran	N/I	97	84	2.01	additive	97	90	0.48	additive
Dinotefuran	I/N	90	84	0.42	additive	93	90	0.07	additive
Dinotefuran	N+I	97	84	2.01	additive	97	90	0.48	additive
Indoxacarb	N/I	73	72	0.01	additive	97	74	7.43	synergistic
Indoxacarb	I/N	47	72	8.68	antagonistic	87	74	2.43	additive
Indoxacarb	N+I	87	72	3.12	additive	97	74	7.43	synergistic
Imidacloprid	N/I	80	85	0.29	additive	63	88	7.30	antagonistic
Imidacloprid	I/N	93	85	0.75	additive	50	88	16.81	antagonistic
Imidacloprid	N+I	97	85	1.60	additive	63	88	7.30	antagonistic

[x]I/N: insecticide applied first, nematode applied 24 h later; N/I: nematode applied first, insecticide applied 24 h later; N+I: nematode and insecticide applied at the same time.
[y]Interaction based on X^2 mortality ratio (expected : observed).
[z]Additive: $X^2 < 3.84$; Synergistic: $X^2 > 3.84$ and $P_C > P_E$; Antagonistic: $X^2 > 3.84$ and $P_C < P_E$, where P_C is the observed mortality of the insecticide and nematode combination and P_E is the expected mortality of the combination.

were higher in cadavers exposed to the simultaneous application of imidacloprid and *S. riobrave* (Fig.3C), but no changes in progeny production were observed with either of the alternate application timings.

DISCUSSION

In this study, we investigated the effect of three insecticides commonly used for pest management in Arizona on two Arizona-native EPN species under laboratory conditions. Specifically, we assessed the survival, virulence, and reproductive fitness of *S. riobrave* (SR5 strain) and *H. sonorensis* (Caborca strain). None of the tested insecticide concentrations significantly affected IJ survival of either nematode species considered in this study. However both EPN species responded differently to the effect of the selected insecticides and timing of application. In particular, virulence of IJs and reproduction of adults were the most affected parameters in the life cycle of nematodes.

Dinotefuran increased the virulence of both EPN species when compared with the application of the nematodes alone or the insecticide alone. Interestingly, dinotefuran did not significantly affect reproduction of *H. sonorensis* as IJ progeny numbers remained about the same when compared to those emerging from the nematode application. Contrastingly, this insecticide favored reproduction of *S. riobrave* as reflected by an increment of the emerging IJ population when compared with that produced by the nematode alone.

Indoxacarb, either applied simultaneously or after EPN, showed a synergistic effect on *S. riobrave* virulence. Indeed, insect mortality increased to 90% or more when *S. riobrave* was applied in combination with this insecticide at the above-mentioned inoculation timings. In contrast, at the same inoculation timings, the interaction of this insecticide with *H. sonorensis* was only considered additive. Mortality of *H. zea* was not significantly different when indoxacarb was applied before the nematode inoculum. However, an increase in larvae mortality was achieved when both *H. sonorensis* and indoxacarb were inoculated simultaneously. An antagonistic effect was observed when indoxacarb was added prior to the inoculation of *H. sonorensis*. Interestingly, none of the nematode-insecticide combination timings significantly affected nematode reproduction as observed by the numbers of IJs produced.

This insecticide has a non-systemic effect that targets the nervous system of the insects by blocking sodium channels and causing paralysis of the larva (Environmental Protection Agency, 2000). Gaugler *et al.* (1994) reported feeding cessation of targeted insects when this insecticide is applied and suggested that this may cause changes in the behavior morphology and physiology of the larvae, making them more susceptible to EPN infection.

A similar scenario could help explain the results of this study. For example, we also noticed that time to death of *H. zea* larvae was augmented with any of the EPN-indoxacarb treatments when compared

with other EPN-insecticide combinations tested. On average, it took 1 to 2 d for *H. zea* larvae to die after exposure to the EPN-indoxacarb treatments and 5 to 7 d for any of the other EPN-insecticide treatments (data not shown). It is possible that indoxacarb had a more rapid debilitating effect on *H. zea* larvae than any of the other insecticides studied and became a more susceptible target for the nematodes to parasitize.

With respect to imidacloprid, previous studies have shown that this insecticide had synergistic interactions when combined with other EPN species such as *H. bacteriophora* and *S. glaseri* (Koppenhöfer *et al*., 2000; Koppenhöfer and Fuzy, 2008). Interestingly, results of this study show opposite outcomes. Specifically, all combinations of *S. riobrave* with imidacloprid had an antagonistic effect. Imidacloprid is a systemic insecticide that targets mostly sucking insects including aphids, thrips, and whiteflies, and also scarab beetles, as well as other insects (National Pesticide Information Center, 2012). We speculate that the antagonistic effects observed in this study may be related to the fact that this insecticide is not suitable for controlling lepidopteran pests such as *H. zea*.

Overall, results from this study show that none of the insecticide-EPN combinations examined have a clear advantage relative to the single application of either the nematodes or the insecticides alone. From all combinations tested, the synergistic effect observed between the interaction of *S. riobrave* and indoxacarb may be worth considering. Research to evaluate their performance in greenhouse and (or) field settings could prove instructive.

It is also interesting to note the contrasting effects observed on nematode virulence and reproduction for the assessed insecticide-EPN treatments. Why would indoxacarb increase virulence of *S. riobrave* but decrease its reproduction? Why would imidacloprid decrease virulence of *S. riobrave* but increase reproduction? These questions certainly warrant further investigation.

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