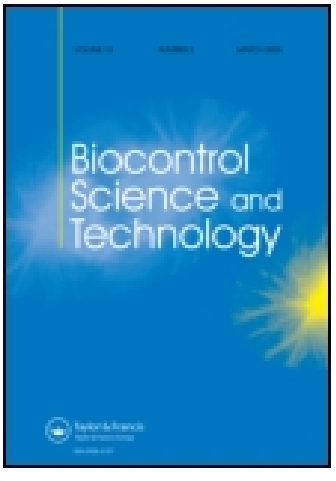

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SHORT COMMUNICATION

Interactions between entomopathogenic nematodes and imidacloprid for rose sawfly control

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Rose sawfly, *Arge ochropus* (Gmelin), is one of the most important pests of ornamental plants such as roses and wild rose bushes in Northern Iran. We investigated the interactions between the insecticides imidacloprid and the entomopathogenic nematodes *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* as control agents of fifth-instar larvae in the laboratory. The larvae were very susceptible to *S. carpocapsae* (LC_{50}: 21 infective juvenile per larva) and *H. bacteriophora* (LC_{50}: 32). Combinations of two imidacloprid rates (LC_{30} and LC_{50}) and four rates of each nematode species (LC_{25}–LC_{75}) were tested. Combinations with the lower imidacloprid rate except for that with the highest *H. bacteriophora* rate caused higher mortality than both respective single-agent treatments. In combination with the higher imidacloprid rate, only one combination with *H. bacteriophora* and two combinations with *S. carpocapsae* caused higher mortality than both respective single-agent treatments. Interactions were generally stronger at the lower imidacloprid rate and were stronger for *S. carpocapsae* (synergistic in seven combinations, additive in one) than for *H. bacteriophora* (synergistic in two, additive in six). Synergistic imidacloprid-*S. carpocapsae* combinations could be a useful tool for the control of *A. ochropus* larvae that would simultaneously control other common pests susceptible to imidacloprid.

Keywords: *Arge ochropus*; entomopathogenic nematodes; biological control; synergism; insect pathology

Introduction

The larvae of the rose sawfly, *Arge ochropus* (Gmelin), can be serious pests of ornamental plants including cultivated roses throughout much of the species range (Smith, 1989). After developing through five larval stages feeding on foliage, the mature larvae move into the soil to pupate. There are four to five generations per year in Northern Iran (Sahragard & Heidari, 2001). Entomopathogenic nematodes (EPNs) can be an effective and environmentally safe alternative to chemical insecticide for insect pest control (Grewal, Ehlers, & Shapiro-Ilan, 2005). Several studies have shown that foliar EPN applications can provide good control of larvae of some sawfly species (Bélair, Vincent, & Chouinard, 1998; Georgis & Hague, 1988;

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Narayanan & Gopalakrishnan, 2003) but require high rates and/or multiple applications, making this control approach expensive. Soil application against mature larvae entering the soil and prepupae and pupae would only suppress damage by the next generation which is not a very feasible approach in a high-value crop situation such as cultivated roses.

EPN are compatible with many chemical insecticides, and some EPN-chemical insecticide combinations may result in additive or synergistic target mortality (Koppenhöfer & Grewal, 2005). Such combinations have been studied extensively for soil insects and were particularly strong with the neonicotinoid insecticide imidacloprid against scarab beetle larvae (Koppenhöfer & Grewal, 2005, and references therein; Koppenhöfer & Fuzy, 2008). Very little is known about such interactions for above-ground insect pests. Imidacloprid-EPN combinations against second-instar sweet potato whitefly, *Bemisa tabaci* Gennadius, resulted in additive target mortality with the EPN species *Steinernema feltiae* (Filipjev) but were antagonistic for *S. carpocapsae* (Weiser) (Cuthbertson, Head, Walters, & Murray, 2003; Cuthbertson, Mathers, Northing, Prickett, & Walters, 2008).

Preliminary studies indicated that *A. ochropus* larvae are susceptible to *S. carpocapsae* and *Heterorhabditis bacteriophora* Poinar (same strains as in this study) but not at a level that would make foliar applications of EPNs alone feasible (HS and JK, unpubl. data). Imidacloprid is used for the control of different insect pests in cultivated roses (e.g., rose aphid, *Macrosiphum rosae* L.) at a time when *A. ochropus* larvae are present on the roses' foliage (HS, pers. observations). Our objective was to determine if imidacloprid and EPNs interact synergistically to provide significantly higher mortality of *A. ochropus* larvae than either agent alone.

Material and methods

During September to November 2012, *A. ochropus* larvae were collected from rose bushes on the University of Guilan (Rasht, Guilan, Iran) campus. They were reared to fifth instars in clear plastic containers with rose leaves as food source in a growth chamber (25 ± 1°C, 70 ± 10% RH, 16:8 h L:D). Because of the timing of imidacloprid application for other pests and the higher EPN susceptibility of larger larvae observed in another sawfly species (Georgis & Hague, 1988), we conducted our study with fifth-instar *A. ochropus* larvae (24 h after moulting). Commercial strains of *S. carpocapsae* (Capsanem®) and *H. bacteriophora* (Larvanem®) (Koppert Biological Systems, Berkel en Rodenrijs, The Netherlands) were reared on late instar greater wax moth, *Galleria mellonella* L., larvae. Emerging infective juvenile (IJ) nematodes were collected from White traps over 7 days and stored in water at 10°C for 7–14 days before use (Stock & Goodrich-Blair, 2012). Technical grade (95% AI) imidacloprid was obtained from Kavosh Kimia Co. (Kerman, Iran). Experiments were conducted in a growth chamber (see above).

To determine larval susceptibility to imidacloprid, 0, 5, 7, 11.5, 19, 30.5 or 50 ng imidacloprid in 1 µl of acetone was applied dorsally to the metathorax of larvae, using a micro-applicator (Burkhard Scientific, Rickmansworth, UK). The larvae were kept in 9-cm diameter Petri dishes. Mortality was recorded 48 hours after treatment.

Larval EPN susceptibility was determined in glass containers (2 × 2 × 1.5 cm) with one layer of filter paper to which 150 µl water was added containing 0 or 3, 8, 11, 32 and 130 *H. bacteriophora* IJs or 5, 9, 11, 21 and 46 *S. carpocapsae* IJs.

One larva was added per container and the container was closed with a ventilated lid. Mortality was recorded after 72 hours.

EPN–imidacloprid interaction was studied in the same arenas as the EPN-alone experiment. The larvae were exposed to the LD_{30} or LD_{50} of imidacloprid alone; the LC_{10}, LC_{25}, LC_{30}, LC_{50} or LC_{75} of *S. carpocapsae* alone; the LC_{10}, LC_{25}, LC_{30}, LC_{50} or LC_{75} of *H. bacteriophora* alone, and the combination of each EPN rate (except for the *S. carpocapsae* LC_{75} and *H. bacteriophora* LC_{30}) with each imidacloprid rate. EPNs were applied first in 150 µl to the filter paper, and imidacloprid was applied topically (see above) 1 hour later. Larval mortality was determined after 72 hours.

All experiments had 10 larvae per treatment and were repeated four times. LD and LC values were calculated using POLO-PC software (LeOra software, 1987). Mortality data in the combination experiment were arcsine square-root transformed and analysed using ANOVA followed by means separation, using Tukey's test (SAS Institute, 2004). Synergistic, additive or antagonistic interactions between agents in the combination treatments were determined using a χ^2 test (Koppenhöfer & Fuzy, 2008). The expected additive proportional mortality M_E for the combinations was calculated by $M_E = M_N + M_I(1 - M_N)$, where M_N and M_I are the observed proportional mortalities caused by nematodes and imidacloprid alone, respectively. The results from a χ^2 test, $\chi^2 = \frac{(M_{NI} - M_E)^2}{M_E}$, where M_{NI} is the observed mortality for the nematode–imidacloprid combinations, were compared to the χ^2 table value for 1 *df*. If the calculated χ^2 value exceeded the table value, a non-additive effect between the two agents was suspected. If $M_{NI} - M_E$ had a positive or a negative value, a significant interaction was considered synergistic or antagonistic, respectively.

Results

No control mortality was observed. The LD_{30} and LD_{50} values for imidacloprid 48 h after topical application were 5.6 (1.6–8.9) and 9.6 (4.8–14.7) ng, respectively [slope: 2.204 ± 0.289; $\chi^2(df) = 9.83(4)$]. Based on overlap of the fiducial limits, virulence did not differ between *S. carpocapsae* and *H. bacteriophora* and was high for both species (Table 1). In the combination experiment, significant differences were detected among treatments ($F \geq 39.23$; $df = 28, 115$; $P < 0.0001$; Table 2). All combinations with the lower imidacloprid rate, except for that with the highest *H. bacteriophora* rate, caused higher mortality than both respective single-agent treatments. In combinations with the higher imidacloprid rate, only one combination

Table 1. Mean number of nematodes required to cause 50% (LC_{50}), 75% (LC_{75}) and 90% (LC_{90}) mortality (95% confidence limits) of fifth-instar *Arge ochropus* larvae after 72 h exposures to the entomopathogenic nematodes *H. bacteriophora* and *S. carpocapsae*.

Treatment	*H. bacteriophora*	*S. carpocapsae*
LC_{50}	32 (16–102)	21 (16–25)
LC_{75}	130 (55–4618)	46 (36–62)
LC_{90}	463 (127–18950)	92 (66–159)
Slope	1.101 ± 0.199	1.953 ± 0.277
$\chi^2(df)$	4.02 (3)	0.119 (3)

Table 2. Interaction between entomopathogenic nematodes and imidacloprid on mortality of fifth-instar *Agre ochropus* larvae in a laboratory experiment.

Treatment[a]	Mortality[b] (%) (±SEM)	χ^2-value[c]	D[d]
$Imi_{5.6}$	39 (±2) efg	–	–
$Imi_{9.6}$	58 (±5) cde	–	–
Hb_3	12 (±7) i	–	–
Hb_8	28 (±5) ghi	–	–
Hb_{11}	38 (±5) efgh	–	–
Hb_{32}	55 (±6) def	–	–
Hb_{130}	78 (±6) abcd	–	–
Sc_5	13 (±5) hi	–	–
Sc_9	25 (±6) ghi	–	–
Sc_{11}	33 (±9) fghi	–	–
Sc_{21}	58 (±3) cdef	–	–
Sc_{46}	80 (±6) abcd	–	–
$Imi_{5.6}$ + Hb_3	70 (±8) bcd	12.11***	24
$Imi_{5.6}$ + Hb_8	73 (±5) abcd	5.02*	17
$Imi_{5.6}$ + Hb_{32}	88 (±3) ab	3.08	15
$Imi_{5.6}$ + Hb_{130}	95 (±3) ab	0.88	9
$Imi_{5.6}$ + Sc_5	70 (±4) abcd	19.91***	31
$Imi_{5.6}$ + Sc_9	73 (±5) abcd	14.71***	28
$Imi_{5.6}$ + Sc_{11}	93 (±5) ab	16.52***	31
$Imi_{5.6}$ + Sc_{21}	98 (±3) a	7.41**	23
$Imi_{9.6}$ + Hb_3	78 (±5) abcd	0.77	7
$Imi_{9.6}$ + Hb_8	83 (±5) abc	0.13	3
$Imi_{9.6}$ + Hb_{32}	90 (±4) ab	1.60	11
$Imi_{9.6}$ + Hb_{130}	98 (±0.3) a	0.53	7
$Imi_{9.6}$ + Sc_5	80 (±4) abcd	4.31*	17
$Imi_{9.6}$ + Sc_9	88 (±5) ab	5.27*	19
$Imi_{9.6}$ + Sc_{11}	90 (±4) ab	4.70*	18
$Imi_{9.6}$ + Sc_{21}	98 (±3) a	2.87	15

[a]Hb, *Heterorhabditis bacteriophora*; Sc, *Steinernema carpocapsae*; Imi, Imidacloprid; numbers indicate the number of IJs per larva for nematodes and ng per larva imidacloprid.
[b]Mortality is corrected for control mortality. Means with the same letter are not significantly different (Tukey's test, $P > 0.05$).
[c]χ^2 test ($df = 1$) was used to determine if observed mortality was significantly different (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$) than expected for no interaction.
[d]D, difference between mortality observed and mortality expected for an additive effect: D significantly greater/smaller than 0 = synergistic/antagonistic interaction.

with *H. bacteriophora* (LC_{50}) and two combinations with *S. carpocapsae* (LC_{25}, LC_{30}) caused higher mortality than both respective single-agent treatments. Interactions were generally stronger at the lower imidacloprid rate and were stronger for *S. carpocapsae* (synergistic in seven, additive in one combination) than for *H. bacteriophora* (synergistic in two, additive in six).

Discussion

Our study shows that *A. ochropus* fifth instars are very susceptible to *H. bacteriophora* and particularly *S. carpocapsae*. Nonetheless, given that the larvae feed exposed on the

foliage, rather than in some more protected location on the plant, it can be expected that with EPN-alone applications, high rates and multiple applications would be necessary for effective control. The strong synergistic interaction between *S. carpocapsae* and imidacloprid could make this combination effective enough to reduce the need for multiple applications and/or the need for high EPN rates. If the combination would be applied when imidacloprid is already applied for the control of other pests, it could be very feasible for integration into existing management programmes. This scenario would also not increase any potential negative effects of imidacloprid on pollinators (e.g., Charles et al., 2014), predators and parasitoids (e.g., Hopwood, Black, Vaughan, & Lee-Mäder, 2013).

We cannot explain why the interaction with imidacloprid is stronger for *S. carpocapsae* than for *H. bacteriophora*, but such differences between EPN species in the degree of interaction have been observed previously (e.g., Koppenhöfer & Fuzy, 2008; Koppenhöfer & Grewal, 2005, and references therein). The weaker interactions at the higher imidacloprid rate are at least in large part due to higher control rates by the higher imidacloprid rate alone which allowed less space for significant improvement in the combinations and is typical for such interactions (Koppenhöfer & Fuzy, 2008; Koppenhöfer & Grewal, 2005, and references therein).

In addition to testing *S. carpocapsae*–imidacloprid combinations under field conditions, future studies should also examine whether the efficacy of *S. carpocapsae* and the combination could be further improved by targeting different stages, i.e., fourth- and third-instar larvae on the foliage. Younger stages may be more susceptible to EPN and/or imidacloprid, resulting in better control even if the interaction between the two agents may turn out to be weaker than for the fifth instar (Koppenhöfer & Fuzy, 2008). If application against younger stages would be at least as effective as against the fifth instar, a wider application window would facilitate coordination with imidacloprid application for the control of other pest.

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