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Effect of the anthranilic diamide insecticide, chlorantraniliprole, on *Heterorhabditis bacteriophora* (Rhabditida: Heterorhabditidae) efficacy against white grubs (Coleoptera: Scarabaeidae)

Albrecht M. Koppenhöfer *, Eugene M. Fuzy

Department of Entomology, Rutgers University, Blake Hall, 93 Lipman Dr, New Brunswick, NJ 08901, USA

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Abstract

Combinations of the anthranilic diamide, chlorantraniliprole, and the entomopathogenic nematode *Heterorhabditis bacteriophora* were tested for control of third-instar white grubs in turfgrass. In greenhouse experiments, the combinations had a synergistic or additive effect on the oriental beetle, *Anomala* (=*Exomala*) *orientalis* mortality, whether chlorantraniliprole was applied 1 week before *H. bacteriophora* or simultaneously. Combinations caused mortality to occur more rapidly than for the single agents. *H. bacteriophora*-chlorantraniliprole combinations had a synergistic effect on mortality of Japanese beetle, *Popillia japonica*, and northern masked chafer, *Cyclocephala borealis*, larvae in greenhouse experiments. Synergistic and additive effects on larval mortality were also observed in field experiments with *A. orientalis* and *P. japonica*. Across all experiments, synergistic interactions (based on a χ^2 test) were observed in 64% of the combinations. Significant control (based on ANOVA) was observed in 12% of the chlorantraniliprole alone treatments and 29% of the *H. bacteriophora* alone treatments, but significant control occurred in 76% of the combination treatments. *H. bacteriophora* progeny production per dead larva recovered in greenhouse experiments did not differ between *H. bacteriophora* alone and the combination treatments. Nematode densities were higher at 28 days after treatment in soil from the combination treatments due to the higher number of larvae succumbing to nematode infection. *H. bacteriophora* and chlorantraniliprole were compatible in tank mixes. Agitation in solution with up to 900 ppm chlorantraniliprole did not affect survival, infectivity, and reproduction of *H. bacteriophora*. *H. bacteriophora*-chlorantraniliprole combinations offer a safe and highly IPM compatible alternative for remedial white grub control.

Keywords: *Anomala orientalis*; *Popillia japonica*; *Cyclocephala borealis*; Entomopathogenic nematodes; Anthranilic diamide; Synergism; Turfgrass

1. Introduction

A complex of white grub species, root-feeding larvae of scarab beetles (Coleoptera: Scarabaeidae), is the most destructive and widespread pest of turfgrasses throughout the eastern United States. Extensive damage to turfgrass can be caused by the large larvae under warm, dry conditions in late summer/early fall and less commonly in spring. In addition, vertebrate predators may damage the turf when foraging for the larvae even at larval densities that by themselves would not cause damage. Important species include the native masked chafers, *Cyclocephala* spp., and the introduced Japanese beetle, *Popillia japonica* Newman, throughout most of the eastern states of the USA. In the Northeast and along the eastern seaboard of the USA, important species also include the introduced oriental beetle, *Anomala* (=*Exomala*) *orientalis* Waterhouse, European chafer, *Rhizotrogus majalis* (Razoumowsky), and Asiatic garden beetle, *Maladera castanea* (Arrow) (Potter, 1998; Alm et al., 1999; Vittum et al., 1999; Koppenhöfer, unpublished data). Most of these white grub species are also pests of nursery stock and various horticultural crops (Potter, 1998; Vittum et al., 1999).

Synthetic insecticides are presently the primary means of managing white grubs in the USA. The loss of many

* Corresponding author. Fax: +1 732 932 7229.
E-mail address: koppenhofer@aesop.rutgers.edu (A.M. Koppenhöfer).

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insecticides for curative white grub control due to the implementation of the Food Quality Protection Act of 1996 (FQPA) and the introduction of new chemistries (e.g., halofenozide and neonicotinoids) with long residual activity and optimal performance against young larvae have led to the wide adoption of preventive applications against white grubs (Potter, 1998; Vittum et al., 1999). For optimal performance, application of these newer insecticides is recommended around peak egg-laying of the important white grub species, i.e., in June and July at the latitude of New Jersey. Because white grub outbreaks are difficult to predict, being localized and sporadic in nature, and eggs and young larvae are difficult to sample, preventive applications are usually made over large areas every year. As a result, applications are expensive, increase the chances of resistance development or enhanced microbial degradation, and, by depriving endemic natural enemies of host/prey, they may increase dependency on chemical control.

Entomopathogenic nematodes (Heterorhabditidae and Steinernematidae) have great potential for curative white grub control and offer an environmentally safe and IPM compatible alternative to synthetic insecticides (Grewal et al., 2005). When used under favorable conditions, well-adapted nematode species/strains such as *Heterorhabditis bacteriophora* Poinar can provide curative *P. japonica* control equal to that of standard insecticides (Georgis and Gaugler, 1991; Grewal et al., 2004). However, other white grub species such as *A. orientalis*, *R. majalis*, *M. castanea*, and May/June beetles (*Phyllophaga* spp.) appear to be more difficult to control (Grewal et al., 2005; Koppenhöfer et al., 2004, 2006). The only species that has provided good control of these species is *Steinernema scarabaei* Stock and Koppenhöfer (Koppenhöfer and Fuzy, 2003; Grewal et al., 2005), a species that thus far has not been possible to mass-produce.

In the absence of highly effective, commercially-available nematode species/strains, combining nematodes with synergists may be a feasible alternative for white grub management. Combinations of nematodes with the bacteria *Paenibacillus popilliae* (Dutky) (Thurston et al., 1994) and *Bacillus thuringiensis* Berliner Buibui strain (Koppenhöfer et al., 1999) have resulted in improved white grub control, but both combinations have various limitations. The most feasible and reliable synergists for nematodes appear to be the neonicotinoid insecticides, particularly imidacloprid. Imidacloprid interacted synergistically with several nematode species resulting in higher mortality of *P. japonica*, *A. orientalis*, and three *Cyclocephala* spp. than with each agent alone, had no negative effects on nematode reproduction in infected larvae, and was compatible with the nematodes in tank mixes (Koppenhöfer et al., 2002, 2003; Koppenhöfer and Grewal, 2005).

The anthranilic diamides are a new class of insecticides that binds to a novel target site, the ryanodine receptor, resulting in the uncontrolled release of calcium stores from the sarcoendoplasmic reticulum (Lahm et al., 2005; Cordova et al., 2006). In the target organism, this causes impaired regulation of muscle contraction and leads to feeding cessation, lethargy, paralysis, and death. Anthranilic diamides have very low vertebrate toxicity due to a >500-fold differential selectivity toward insect over mammalian receptors (Cordova et al., 2006). The anthranilic diamide chlorantraniliprole has shown high efficacy against various turfgrass pests including the white grub complex (Koppenhöfer, unpublished data). It can be applied as early as April for preventive white grub control due to its long residual activity; however, it is not effective for curative white grub control (Koppenhöfer, unpublished data).

The objective of this study was to determine the potential of combined applications of the entomopathogenic nematode *H. bacteriophora* and chlorantraniliprole for curative control of different white grub species. Because nematodes can recycle in hosts after inundative applications (Klein and Georgis, 1992), thereby increasing nematode persistence and grub control, we also tested the compatibility of nematodes and chlorantraniliprole with respect to nematode reproduction. Finally, we tested the compatibility in tank mixes as application in one spray mix would further increase the ease of use for applicators.

2. Materials and methods

2.1. General methods

Third-instar larvae collected in late September/early October were used in all greenhouse experiments. *A. orientalis*, *P. japonica*, and *Cyclocephala borealis* arrow were collected in turf areas at the Rutgers University Research Farms (Adelphia, NJ; North Brunswick, NJ). None of the sites had been treated with insecticides during the previous year. The larvae were kept individually in the cells of 24-well plates in sandy loam at 15 °C for short-term storage and at 10 °C for long term storage (1–10 weeks). The larvae were returned to room temperature (21–24 °C) for 24 h before use in experiments. *H. bacteriophora* (GPS11 strain) was cultured in last instars of the greater wax moth, *Galleria mellonella* (L.). The emerging infective juveniles (IJs) were harvested over a period of 10 days from emergence traps, i.e., modified White traps (Kaya and Stock, 1997), and stored in tap water at 10 °C for 6–21 days before use. The soil used in the greenhouse experiments was a sandy loam (61% sand, 27% silt, 12% clay, 2.3% organic matter, pH 5.9) that had been pasteurized (3 h at 70 °C) and air-dried before use. Chlorantraniliprole was obtained as a flowable liquid formulation with 18.5% active ingredient (ai) (DuPont Ag Products, Wilmington, DE) and trichlorfon (Dylox 80) as a wettable powder with 80% ai (Bayer Environmental Science, Montvale, NJ).

2.2. Greenhouse experiments

To determine the effect of chlorantraniliprole on *H. bacteriophora* efficacy against and reproduction in third instars

of different white grub species, a series of greenhouse experiments was conducted. One-liter pots (10 cm × 10 cm × 10 cm; 100 cm^2 at soil surface) filled with sandy loam to a height of 9 cm were seeded with perennial ryegrass, *Lollium perenne* L., and watered every 2–3 days until the end of the experiment. The grass was allowed to grow for 4 weeks and then cut using scissors before introduction of 5 larvae/pot. The larvae were placed on the grass 3 days before the start of an experiment. Larvae that had not entered into the soil within 24 h were replaced. The greenhouse was maintained at 28 °C/18 °C (day/night; 14/10 h L/D) and the soil temperature in the pots averaged 23.0 ± 1.5 °C. Treatments were applied in 50 ml of water. Controls received 50 ml water only. After application, pots were arranged in a completely randomized design.

2.2.1. Effect on larval mortality

The first experiment evaluated the effect of timing of chlorantraniliprole application relative to *H. bacteriophora* application on *A. orientalis* mortality. All pots were infested with larvae at the same time and destructively sampled 24 days later. A first set of pots was treated with chlorantraniliprole (100, 200, or 400 g active ingredient (ai)/ha) 3 days after release of larvae. Half of this first set of pots for each chlorantraniliprole rate was treated with *H. bacteriophora* (500 IJs/pot = 0.5×10^9 IJs/ha) 10 days after release of larvae; the other half received water only. A second set of pots received chlorantraniliprole (100, 200, or 400 g ai/ha) 10 days after release of larvae (7 days after the first set of pots). Half of the pots of this second set for each chlorantraniliprole rate was concurrently treated with *H. bacteriophora* (500 IJs/pot = 0.5×10^9 IJs/ha), the other received water only (see also Fig. 1 for treatments). There were seven pots for each combination of chlorantraniliprole rate, chlorantraniliprole application timing, and addition or not of *H. bacteriophora*. Ten pots each received water only on both application dates (control) or water at 3 days and *H. bacteriophora* at 10 days after larval release.

In the second to fourth experiment, all treatments were applied at the same time and evaluated at 14 days after treatment (DAT). There were 10 replicates for the control and for each treatment. The second experiment evaluated combinations of different chlorantraniliprole and *H. bacteriophora* rates against *A. orientalis*. Treatments were (1 and 2) chlorantraniliprole alone at 200 g or 400 g/ha, (3 and 4) *H. bacteriophora* alone at 0.5×10^9 or 1.0×10^9 IJs/ha, and (5–8) the combination of each chlorantraniliprole rate with each *H. bacteriophora* rate. In experiment three, chlorantraniliprole (300 g ai/ha), *H. bacteriophora* (0.2×10^9 IJs/ha), and their combination were tested against *P. japonica*. In experiment four, chlorantraniliprole (300 g ai/ha), *H. bacteriophora* (1.0×10^9 IJs/ha), and their combination were tested against *C. borealis*. The fifth experiment examined the effect of chlorantraniliprole (300 g ai/ha), *H. bacteriophora* (1.0×10^9 IJs/ha), and their combination on mortality of *A. orientalis* over time. Ten pots per treatments were evaluated each at 7, 14, 21, and 28 DAT. All experiments were conducted twice.

2.2.2. Effect on nematode reproduction

In the first four greenhouse experiments, larvae that were recovered intact and with signs of *H. bacteriophora* infection in the treatments containing *H. bacteriophora* were carefully rinsed with tap water and placed individually on emergence traps. The emerging IJs were collected over a 2-week period and stored in tissue culture flasks at 10 °C until counting could be done. The number of progeny per cadaver with signs of *H. bacteriophora* infection was determined by counting four subsamples under a dissecting microscope.

In the fifth experiment, nematode populations in the pots were determined using "saturation baiting" with wax moth larvae (Koppenhöfer et al., 1997). After removal of the larvae, the soil from each pot was mixed after evaluation and a 100 g subsample was placed in a 25 × 100 mm petri dish. The soil moisture was adjusted to approximately 13% by adding water or letting the soil air dry for a short period. Five wax moth larvae were added per dish. Every 3 days, dead larvae were replaced with new larvae and baiting continued until no more infected larvae were found for two consecutive 3-day periods. Nematode infection was determined by the presence or absence of the typical orange to red color of *H. bacteriophora*-infected wax moth larvae.

2.3. Field experiments

Two similar field experiments were conducted at the Rutgers University Research Farm (Adelphia, NJ) in 4–5-year old turfgrass areas planted with tall fescue (*Festuca arundinacea* Schreb.) and maintained using typical management procedures for intermediate turf maintenance levels. Mowing height was 3.8 cm and the thatch layer was 23 mm thick. The soils were sandy loams (65–69% sand, 18–21% silt, 13–16% clay, 2% organic matter, pH 6.5–6.8). Preapplication sampling showed that the sites had low resident white grub populations (5–10 larvae/m^2) consisting of about 60% *P. japonica* and 40% *A. orientalis*. The larvae were about 10% second instar and 90% third instar at the time of application. To increase the white grub populations, larvae collected from adjacent tall fescue areas were released into each replicate by uniformly placing them into the grass at least 10 cm inside from the replicates borders. Larvae that did not burry into the ground within 20 min were replaced. No natural entomopathogenic nematode population was detected by baiting soil samples with greater wax moth larvae. Replicates consisted of 61 × 61 cm turf areas separated from each other by 61 cm in both directions. Experiments were evaluated by determining the number of alive larvae in ten samples per replicate taken with a standard size golf hole cutter (0.01 m^2) to a depth of 7.5 cm. Larvae were identified to species using the raster pattern on the lower side of their abdomen (Potter, 1998).

Three days before treatment application, 18 *A. orientalis* larvae were released into each replicate in the first experiment, and 15 *P. japonica* larvae were release into each replicate in the second experiment. Treatments for both experiments were applied on September 15, 2006 at 1600–1800 h (soil temperature at 5 cm depth 22 °C; air temperature 21 °C; overcast) in 2 l of water per plot (=5 mm) using a watering can. Because the soil was already moist before application, no additional irrigation was applied. There were five replicates per treatment arranged in a randomized complete block design. In the first experiment, treatments were (1–2) chlorantraniliprole at 0.125 or 0.25 kg ai/ha, (3–4) *H. bacteriophora* at 6.25×10^9 or 12.5×10^9 IJs/ha, (5–8) the combination of each chlorantraniliprole rate with each *H. bacteriophora* rate, and (9) trichlorfon (8.2 kg ai/ha). In the second experiment, treatments were (1) chlorantraniliprole at 0.2 kg ai/ha, (2–3) *H. bacteriophora* at 3.125×10^9 or 6.25×10^9 IJs/ha, and (4–5) the combination of chlorantraniliprole with each *H. bacteriophora* rate. Controls received water only. Both experiments were evaluated on October 8 (23 DAT). During the experimental period, the average air temperature was 17.5 ± 0.7 °C. There was a total of 66 mm of rainfall and 20 mm overhead irrigation.

2.4. Tank mix compatibility

To determine whether *H. bacteriophora* and chlorantraniliprole were compatible in tank mixes, we determined IJ survival, infectivity, and reproduction after suspension in chlorantraniliprole solutions. Suspensions of *H. bacteriophora* (500 IJs/ml) containing 0, 100, 300, or 900 ppm (mg ai per liter) chlorantraniliprole were filled in 50-ml Erlenmeyer flasks (20 ml per flask) and agitated on a rotary shaker at 80 rpm for 24 h. Then the IJs were separated from the chlorantraniliprole by pouring the suspension through a sieve (No. 635; 20 μm openings) followed by an additional 500 ml of tap water. The IJs were washed from the sieve into a clean flask and resuspended in 20 ml of tap water. IJ survival (percentage of IJs alive in sample) was determined in six 50 μl samples per flask using a dissecting microscope. Immobile IJs were touched and rolled with a probe and considered dead if they did not react. There were three flasks for each chlorantraniliprole concentration.

To determine IJ infectivity and reproduction after tank mixing, 25 living IJs and one wax moth larva were added to a 30-ml plastic cup filled with 10 g moist sandy loam (12% w/w; −20 kPa water potential). There were 20 cups for each flask. Wax moth larva mortality was observed after 5 days. Dead larvae from the first ten cups were dissected and digested in a pepsin solution to determine the number of nematodes established in them (Mauleon et al., 1993). Dead larvae from the other ten cups were placed individually on emergence traps to determine the number of progeny per cadaver. Emerging IJs were collected over a 2-week period, and stored at 10 °C until counting. The number of progeny per cadaver was determined by counting six subsamples under a dissecting microscope. Infectivity data and progeny data were averaged across the respective ten wax moth larvae (zeros for surviving larvae) so that each flask represented one data point. The experiment was repeated with a different cohort of nematodes.

2.5. Statistical analysis

To normalize data before analysis, larval mortality data (in field experiments but not in greenhouse experiments), nematode progeny from the white grub cadavers (first to fourth greenhouse experiment), and number of wax moth larvae infected with *H. bacteriophora* (fifth greenhouse experiment) were square root transformed, whereas percent mortality of IJs and wax moth larvae (tank mix compatibility experiment) were arcsine square root transformed. The data were subjected to analysis of variance (ANOVA) with experiment repetition, sampling date, and treatment rate as factors, as appropriate for each experiment. Means were separated using Tukey's test. Synergistic, additive, or antagonistic interactions between agents in the combination treatments were determined using a χ^2 test (Finney, 1964; McVay et al., 1977; Koppenhöfer et al., 1999). Grub mortality was calculated by subtracting the number of surviving grubs from the number of grubs released for each replicate and correcting for control mortality (Abbott, 1925). In the field experiment, grub mortality was calculated by dividing the number of detected grubs in a treatment by the mean number of grubs recovered in the control. The expected additive proportional mortality M_E for the nematode-chlorantraniliprole combinations was calculated by $M_E = M_N + M_C(1 - M_N)$, where M_N and M_C are the observed proportional mortalities caused by nematodes and chlorantraniliprole alone, respectively. Results from a χ^2 test, $\chi^2 = (M_{NC} - M_E)^2/M_E$, where M_{NC} is the observed mortality for the nematode-chlorantraniliprole 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 (Finney, 1964). If the difference $M_{NC} - M_E$ had a positive value, a significant interaction was considered synergistic. If the difference had a negative value, a significant interaction was considered antagonistic. Differences among means in all experiments were considered significant at $P < 0.05$. Means ± SE are presented.

3. Results

3.1. Greenhouse experiments

3.1.1. Effect on larval mortality

In the first experiment, *A. orientalis* mortality was significantly affected by treatment ($F = 12.15$; df = 13, 90; $P < 0.0001$) (Fig. 1). *H. bacteriophora* alone and all chlorantraniliprole alone treatments did not cause significant

mortality. All combination treatments caused significant mortality. The combinations of *H. bacteriophora* with the 200 g and 400 g ai/ha rates caused higher mortality than the chlorantraniliprole alone treatments. And the combination with 400 g ai/ha chlorantraniliprole when applied simultaneously caused higher mortality than *H. bacteriophora* alone. *H. bacteriophora* interacted synergistically with the 200 g and 400 g ai/ha rates of chlorantraniliprole, whether applied separately or simultaneously ($\chi^2 \geqslant 16.77$; df = 1; $P \leqslant 0.001$). The interaction with the 100 g ai/ha rate was additive. The chlorantraniliprole alone and the combination treatments followed the same trend whether chlorantraniliprole was applied 7 or 0 days before *H. bacteriophora*. When data were analyzed with the control and *H. bacteriophora* alone treatments excluded, there was no significant effect of chlorantraniliprole rate or application time, and no interaction between rate and time.

In the second experiment, *A. orientalis* mortality was significantly affected by chlorantraniliprole rate ($F = 15.17$; df = 2, 81; $P < 0.0001$) and *H. bacteriophora* rate ($F = 51.76$; df = 2, 81; $P < 0.0001$), but there was no interaction between chlorantraniliprole and *H. bacteriophora* rate (Fig. 2). *H. bacteriophora* alone at the higher rate and all combinations had significantly higher mortality than the control. Among the combinations only that of the higher *H. bacteriophora* rate with the lower chlorantraniliprole rate caused significantly higher mortality than both respective single agent treatments. The higher *H. bacteriophora* rate interacted synergistically with both chlorantraniliprole rates ($\chi^2 \geqslant 6.54$; df = 1; $P \leqslant 0.02$). The interaction of the lower *H. bacteriophora* rate with both chlorantraniliprole rates was additive.

In the third experiment, *P. japonica* mortality was significantly affected by treatment ($F = 110.14$; df = 3, 72; $P < 0.0001$) but not by experiment repetition, and there was no interaction between treatment and experiment repetition. Mortality was significantly higher in the combination treatment than in the *H. bacteriophora* alone and chlorantraniliprole alone treatments which in turn had higher mortality than the control (Fig. 3). *H. bacteriophora* interacted synergistically with chlorantraniliprole ($\chi^2 = 32.7$; df = 1; $P < 0.001$).

In the fourth experiment, *C. borealis* mortality was significantly affected by treatment ($F = 88.88$; df = 3, 72; $P < 0.0001$) but not by experiment repetition, and there was no interaction between treatment and experiment repetition. Mortality was significantly higher in the combination treatment than in all other treatments and also significantly higher for *H. bacteriophora* alone than in the control (Fig. 3). *H. bacteriophora* interacted synergistically with chlorantraniliprole ($\chi^2 = 156.7$; df = 1; $P < 0.001$).

In the fifth experiment, data were first analyzed uncorrected and including the control. For all data combined, there were significant interactions on *A. orientalis* mortality between treatment and experiment repetition ($F = 9.73$; df = 3, 297; $P < 0.001$) and treatment and sampling date ($F = 4.33$; df = 9, 297; $P < 0.001$). When data were analyzed by sampling date, there were significant interactions between experiment repetition and treatment on days 14 to 28 ($F \geqslant 2.79$; df = 3, 72; $P \leqslant 0.047$). Thus, data were analyzed by experiment repetition. In the first experiment repetition, there was a significant interaction between treatment and sampling date ($F = 5.31$; df = 9, 144; $P < 0.001$). At 7 DAT, the combination caused significantly higher mortality than all other treatment, but the single agents did not cause significant mortality ($F = 32.29$; df = 3, 36; $P < 0.001$). At 14 DAT, the combination had significantly higher mortality than *H. bacteriophora* which had significantly higher mortality than chlorantraniliprole which had significantly higher mortality than the control

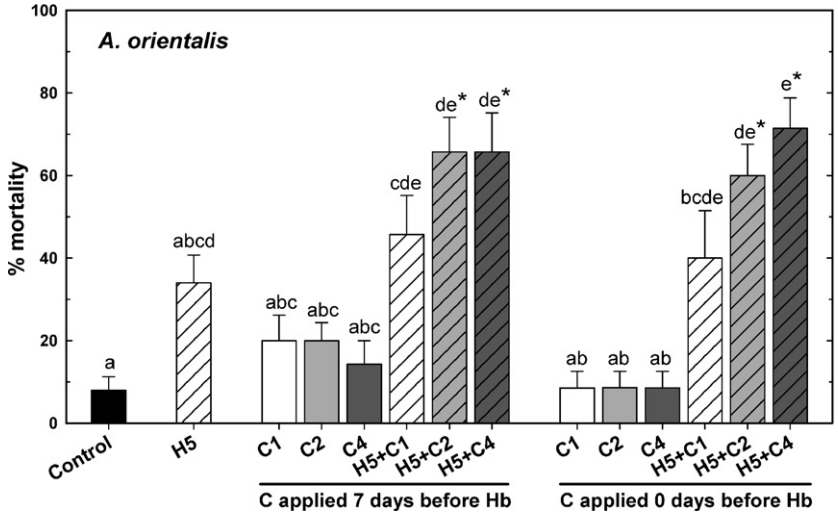

Fig. 1. Effect of application interval on the efficacy of combinations of *Heterorhabditis bacteriophora* (H5; 5×10^8 IJs/ha) and chlorantraniliprole (C; numbers are rates in 0.1 kg ai/ha) against third-instar *Anomala orientalis* in 1-l pots with grass. Chlorantraniliprole was applied 7 days before or at the same time as *H. bacteriophora*, and larval mortality was evaluated 14 days after *H. bacteriophora* application. Means (±SE) with same letter are not significantly different ($P < 0.05$). An '*' indicates significant synergistic interaction between *H. bacteriophora* and chlorantraniliprole.

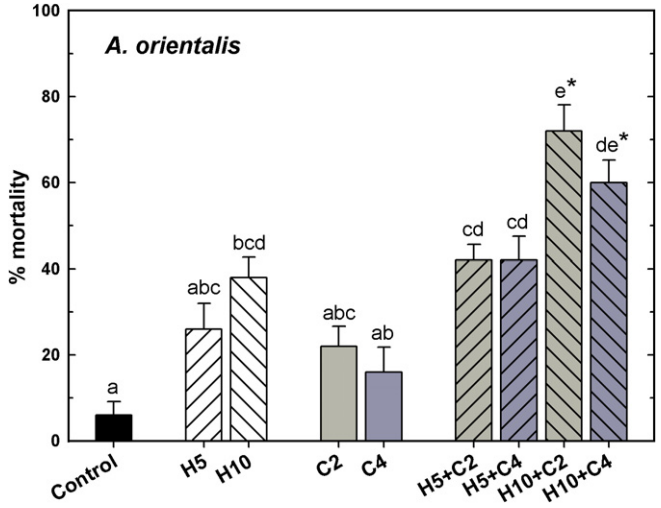

Fig. 2. Effect of combinations of *Heterorhabditis bacteriophora* (H; numbers are rates at 10^8 IJs/ha) and chlorantraniliprole (C; numbers are rates in 0.1 kg ai/ha) on mortality of third-instar *Anomala orientalis* in 1-l pots with grass at 14 days after application. Means (±SE) with same letter are not significantly different ($P < 0.05$). An '*' indicates significant synergistic interaction between *H. bacteriophora* and chlorantraniliprole.

($F = 62.76$; df = 3, 36; $P < 0.001$). At 21 and 28 DAT, the combinations caused significantly higher mortality than *H. bacteriophora* and chlorantraniliprole, and the control had lower mortality than all treatments ($F \geqslant 54.12$; df = 3, 36; $P < 0.001$). In the second experiment repetition, there was no significant interaction between sampling date and treatment. Mortality was significantly lower at 7 DAT than at 14 DAT and was the highest at 21 and 28 DAT ($F = 28.26$; df = 3, 144; $P < 0.001$). Across all sampling dates, the combination had significantly higher mortality

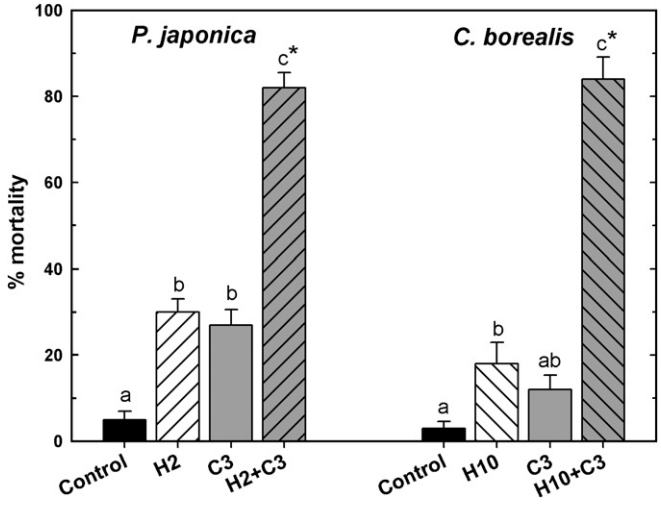

Fig. 3. Effect of combinations of *Heterorhabditis bacteriophora* (H10; 10^9 IJs/ha) and chlorantraniliprole (C3; 0.3 kg ai/ha) on mortality of third-instar *Popillia japonica* and *Cyclocephala borealis* in 1-l pots with grass at 14 days after application. Means (±SE) with same letter are not significantly different within white grub species ($P < 0.05$). An '*' indicates significant synergistic interaction between *H. bacteriophora* and chlorantraniliprole.

than *H. bacteriophora* which had significantly higher mortality than chlorantraniliprole which had significantly higher mortality than the control ($F = 78.56$; df = 3, 144; $P < 0.001$).

To simplify comparisons among treatments and sampling dates in the fifth experiment, *A. orientalis* mortality data were also analyzed after control-correction (Abbott, 1925). For all data combined there was a significant effect of sampling date ($F = 44.07$; df = 3, 222; $P < 0.001$) and treatment ($F = 136.58$; df = 2, 222; $P < 0.001$) but not of experiment repetition, and there were no interactions between sampling date, treatment, or experiment repetition. Corrected mortality was significantly higher at 21 and 28 DAT than at 14 DAT and was the lowest at 7 DAT. Mortality was significantly higher in the combination than for *H. bacteriophora* and was the lowest for chlorantraniliprole (Fig. 4). The combination caused the highest mortality on each sampling date. Mortality increased significantly over time in all treatments but the increase was the fastest for the combination and the slowest for chlorantraniliprole alone. *H. bacteriophora* interacted synergistically with chlorantraniliprole at 7 DAT ($\chi^2 = 19.86$; df = 1; $P < 0.001$), 14 DAT ($\chi^2 = 7.53$; df = 1; $P < 0.01$), 21 DAT ($\chi^2 = 3.98$; df = 1; $P < 0.05$), and 28 DAT ($\chi^2 = 6.24$; df = 1; $P < 0.02$).

3.1.2. Effect on nematode reproduction

H. bacteriophora progeny production (IJs per recovered cadaver) was not significantly affected by trial, treatment, chlorantraniliprole rate, *H. bacteriophora* rate, or timing of chlorantraniliprole application relative to *H. bacteriophora* application in any of the experiments. Average progeny production per cadaver in the treatments ranged from 11,118 to 30,741 for *A. orientalis*, 11,220 to 11,249 for *P. japonica*, and 19,632 to 39,460 for *C. borealis* (Table 1).

The number of wax moth larvae infected by *H. bacteriophora* in soil samples from the fifth experiment was significantly affected by sampling date ($F = 14.80$; df = 3, 136; $P < 0.001$) but not by experiment repetition or treatment. Interactions between experiment repetition and sampling date or experiment repetition and treatment were not significant, but there was a significant interaction between sampling date and treatment ($F = 8.64$; df = 3, 136; $P < 0.001$). Thus, there was no significant effect of treatment at 7–21 DAT. But at 28 DAT, more than three times as many wax moth larvae were infected in the combination treatment than in the *H. bacteriophora* alone treatment ($F = 34.05$; df = 1, 34; $P < 0.001$) (Fig. 4).

3.2. Field experiments

In the first experiment, the average number of white grubs recovered from the untreated plots at evaluation (23 DAT) was 4.4 ± 0.5 *A. orientalis* per 0.1 m^2 and 4.0 ± 0.6 *P. japonica* per 0.1 m^2 with 100% of both species being third instars. *A. orientalis* recovery differed significantly among treatments ($F = 7.41$; df = 9, 36;

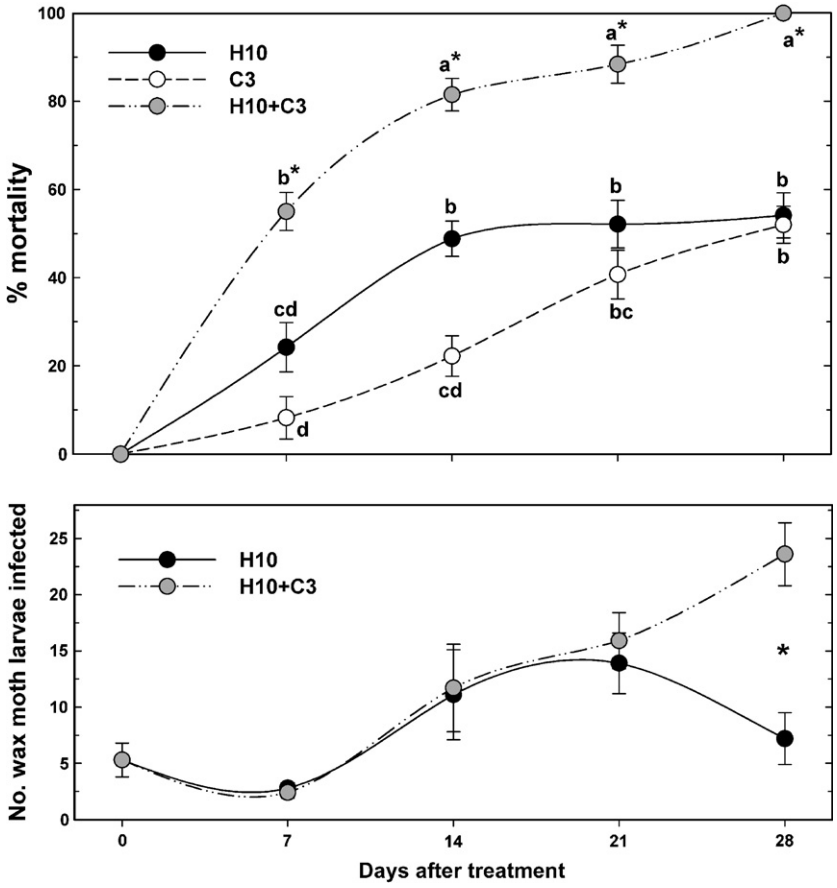

Fig. 4. Effect of combinations of *Heterorhabditis bacteriophora* (H; numbers are rate at 10^8 IJs/ha) and chlorantraniliprole (C3; 0.3 kg ai/ha) on mortality of third-instar *Anomala orientalis* (top) and *H. bacteriophora* population in soil (measured as number of wax moth larvae infected in soil samples) (bottom) in 1-l pots with grass at 0–28 days after treatment. Means (±SE) with same letter are not significantly different ($P < 0.05$). An '*' indicates significant synergistic interaction between *H. bacteriophora* and chlorantraniliprole (top) or significant difference ($P < 0.05$) between means within sampling date (bottom).

Table 1
Effect of chlorantraniliprole on *Heterorhabditis bacteriophora* reproduction in third-instars of *Anomala orientalis*, *Popillia japonica*, and *Cyclocephala borealis*

Experiment—species	Treatment [a]	IJs per larva ± SE[b] (*n*)
1—*A. orientalis*	H5	30,073 ± 5581 (7)
	H5 + C1	30,741 ± 6354 (18)
	H5 + C2	27,396 ± 5649 (27)
	H5 + C4	23,215 ± 4785 (29)
2—*A. orientalis*	H5	22,632 ± 6248 (7)
	H5 + C2	14,649 ± 3852 (15)
	H5 + C4	13,638 ± 2239 (11)
	H10	17,761 ± 7702 (7)
	H10 + C2	11,118 ± 2873 (23)
	H10 + C4	17,012 ± 4602 (16)
3—*P. japonica*	H2	11,249 ± 2944 (14)
	H2 + C3	11,220 ± 2313 (34)
4—*C. borealis*	H10	19,632 ± 4744 (11)
	H10 + C3	39,460 ± 5442 (56)

[a] H, *Heterorhabditis bacteriophora* (numbers are rates at 10^8 IJs/ha); C, chlorantraniliprole (numbers are rates in 0.1 kg ai/ha).

[b] Infective juvenile (IJs) numbers per recovered cadaver did not differ significantly among means in any experiment. '*n*' is the number of recovered cadavers per treatment.

$P < 0.001$). Only trichlorfon and the combinations with the higher chlorantraniliprole rate and the combination of the higher *H. bacteriophora* rate with the lower chlorantraniliprole rate caused significant mortality (Fig. 5). However, only the combination of the higher chlorantraniliprole rate with the lower *H. bacteriophora* rates caused mortality higher than both of the respective single agent treatments. The χ^2 test showed an additive effect on *A. orientalis* mortality of the combination of the higher rates of both agents ($\chi^2 = 2.92$; df = 1; $P = 0.09$) but a synergistic interaction for all other combinations ($\chi^2 = 4.45$; df = 1; $P \leqslant 0.03$).

Recovery of *P. japonica* followed a similar trend as for *A. orientalis* across the treatments ($F = 3.00$; df = 9, 36; $P < 0.01$) with the exception of trichlorfon causing lower mortality. However, only the combination of the higher chlorantraniliprole rate and higher *H. bacteriophora* rate caused significant mortality (Fig. 5). None of the combinations caused significantly higher mortality than both of the respective single agent treatments. The χ^2 test showed an additive effect on *P. japonica* mortality of the combinations with the higher chlorantraniliprole rate ($\chi^2 \leqslant 3.46$; df = 1; $P \geqslant 0.07$) but showed a synergistic interaction for the

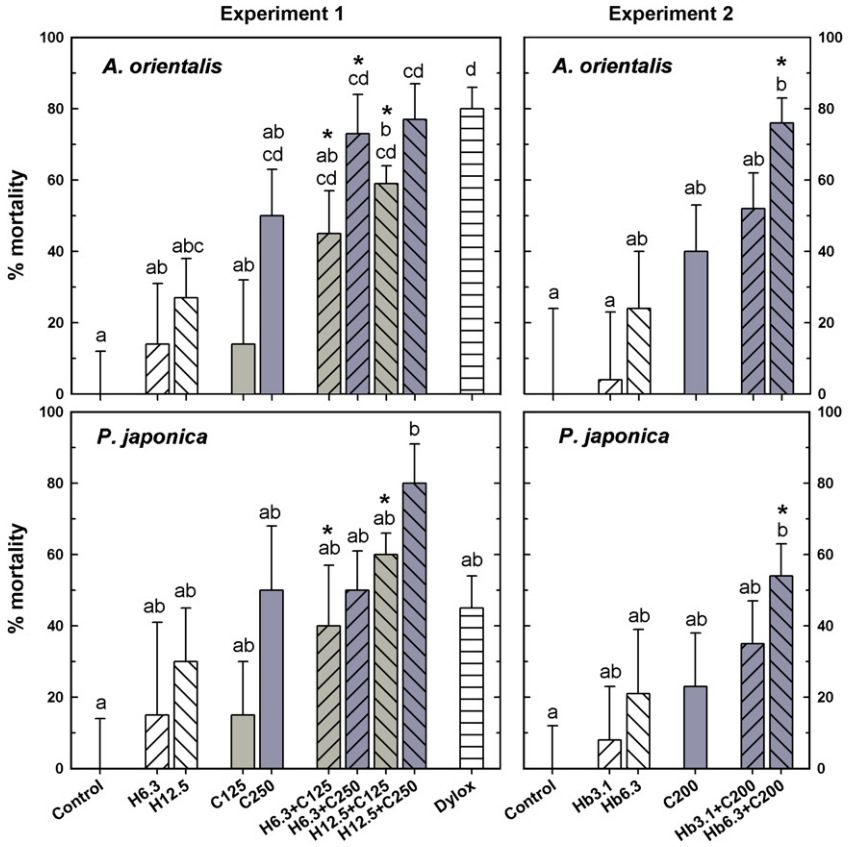

Fig. 5. Effect of combinations of *Heterorhabditis bacteriophora* (H; numbers are rates at 10^8 IJs/ha) and chlorantraniliprole (C; numbers are rates in g ai/ha) on mortality of third-instar *Anomala orientalis* (top graphs) and *Popillia japonica* (bottom graphs) in two field experiments (left vs. right graphs) at 23 days after application. Endemic white grub populations in the experimental plots were increased by releasing larvae collected from adjacent turfgrass areas. Means (±SE) with same letter are not significantly different ($P < 0.05$). An '∗' indicates significant synergistic interaction between *H. bacteriophora* and chlorantraniliprole.

combinations with the lower chlorantraniliprole rate ($\chi^2 = 5.41$; df = 1; $P \leqslant 0.02$).

In the second experiment, the average number of white grubs recovered from the untreated plots at evaluation (23 DAT) was 5.0 ± 1.2 *A. orientalis* per 0.1 m^2 and 9.6 ± 1.1 *P. japonica* per 0.1 m^2 with 100% of both species being third instars. *A. orientalis* recovery was significantly reduced ($F = 3.45$; df = 5, 20; $P < 0.05$) only in the combination of chlorantraniliprole with the higher *H. bacteriophora* rate (Fig. 5). No combination treatment caused mortality higher than both of the respective single agent treatments. The χ^2 test showed an additive effect on *A. orientalis* mortality of the combination with the lower *H. bacteriophora* rate ($\chi^2 = 2.17$; df = 1; $P = 0.12$) but a synergistic interaction for the combinations with the higher *H. bacteriophora* rate ($\chi^2 = 8.58$; df = 1; $P < 0.001$).

Recovery of *P. japonica* followed a similar trend as for *A. orientalis* across the treatments with a significant reduction only in the combination of chlorantraniliprole with the higher *H. bacteriophora* rate ($F = 3.00$; df = 9, 36; $P < 0.01$) (Fig. 5). No combination treatment caused mortality higher than both of the respective single agent treatments. The χ^2 test showed an additive effect on *P. japonica* mortality of the combination with the lower *H. bacteriophora* rate ($\chi^2 = 1.26$; df = 1; $P = 0.25$) but a synergistic interaction for the combinations with the higher *H. bacteriophora* rate ($\chi^2 = 5.92$; df = 1; $P < 0.05$).

3.3. Tank mix compatibility

None of the measured *H. bacteriophora* parameters was significantly affected by tank mixing with chlorantraniliprole ($F \leqslant 1.42$; df = 3, 16; $P \geqslant 0.27$). Percentage IJ survival after 24 h in suspension was 99.0 ± 0.7, 98.3 ± 1.5, 96.7 ± 1.0, and 87.3 ± 3.0 for 0, 100, 300, or 900 ppm chlorantraniliprole, respectively. Percentage wax moth mortality after 5 days exposure to 25 IJs was 88 ± 4, 87 ± 6, 90 ± 3, and 94 ± 2 for 0, 100, 300, and 900 ppm, respectively. The average number of *H. bacteriophora* established per wax moth larva was 3.3 ± 0.7, 2.5 ± 0.4, 4.2 ± 1.1, and 4.3 ± 0.6 for 0, 100, 300, and 900 ppm, respectively. And the average number of progeny per wax moth larva was $197{,}548 \pm 9187$, $199{,}829 \pm 12{,}760$, $195{,}724 \pm 9323$, and $204{,}120 \pm 15{,}830$ IJs for 0, 100, 300, and 900 ppm, respectively.

4. Discussion

Our observations show that *H. bacteriophora* and chlorantraniliprole can be combined for improved curative

white grub control without negative effects on nematode reproduction in infected larvae. Whether chlorantraniliprole was applied 1 week before *H. bacteriophora* or simultaneously, the interactions ranged from an additive to a synergistic effect on mortality of *A. orientalis* (six combinations additive; 12 synergistic), *P. japonica* (three additive; four synergistic), and *C. borealis* (one synergistic). Overall, synergistic interaction was observed in 16 out of 25 combinations. The combinations caused significantly higher mortality than both of the respective single agent treatments only in five out of 25 combinations but clearly provided more consistent control than the single agents. Thus, significant suppression was observed in two out of 17 chlorantraniliprole alone treatments, and four out of 14 *H. bacteriophora* alone treatments, but occurred in 19 out of 25 combinations. In addition, combinations also caused mortality quicker than the single agents, an important benefit given that damage by larger white grubs can occur very rapidly.

Overall, our observations on *H. bacteriophora*-chlorantraniliprole combinations are similar to those on *H. bacteriophora*-imidacloprid combinations (e.g., Koppenhöfer et al., 2000, 2002; Koppenhöfer and Grewal, 2005). Both combinations had additive or, more often, synergistic effects on white grub mortality. Imidacloprid also interacted synergistically with other entomopathogenic nematode species [i.e. *H. megidis* Poinar, Jackson and Klein, *H marelatus* Liu and Berry, *S. feltiae* (Filipjev), *S. scarabaei*, and particularly *S. glaseri* (Steiner) (Koppenhöfer et al., 2000, 2002; Koppenhöfer and Fuzy, 2003)]. Whether chlorantraniliprole interacts synergistically with nematode species other than *H. bacteriophora* needs to be tested.

We did not study the mechanism responsible for the interaction between chlorantraniliprole and *H. bacteriophora*. Imidacloprid disrupts the normal defensive and evasive behaviors that white grubs display in response to entomopathogenic nematode attack and thereby increases the white grubs' nematode-susceptibility (Koppenhöfer et al., 2000). Because chlorantraniliprole appears to have a similar effect on the target organism as imidacloprid including feeding cessation, lethargy, and paralysis (Lahm et al., 2005; Cordova et al., 2006), it is likely that it increases the white grubs' nematode susceptibility in a similar fashion.

Reproduction of *H. bacteriophora* in infected *A. orientalis*, *P. japonica*, and *C. borealis* was not affected by combination with chlorantraniliprole. As the combination treatments generally had two to five times higher mortality than the *H. bacteriophora* alone treatments, nematode numbers in the soil once progeny starts to emerge from the initially infected larvae should also be much higher and stay so for longer periods of time in combination treatments. This is confirmed by the 3-fold higher number of nematode-infected wax moth larvae in soil from combination treatments at 28 DAT in the fifth greenhouse experiment. Similarly, imidacloprid and thiamethoxam had no negative effect on nematode reproduction in white grubs, and imidacloprid, but not thiamethoxam, increased nematode densities in soil in a greenhouse experiment (Koppenhöfer et al., 2003). It is therefore possible that the efficacy of combinations would increase over time because nematode progeny emerging from infected larvae could seek out and kill additional larvae. However, at least in the case of *H. bacteriophora*-imidacloprid combinations, *A. orientalis* and *P. japonica* mortality was not significantly higher in combinations applied in late August than in combinations applied in mid-September (Koppenhöfer and Fuzy, in press).

Entomopathogenic nematodes are compatible in tank mixes with many pesticides including numerous chemical and biological insecticides (Koppenhöfer and Grewal, 2005). Our study shows that chlorantraniliprole can be added to the list of compatible insecticides as 24 h exposure to up to 900 ppm of chlorantraniliprole did not affect *H. bacteriophora* survival, infectivity, and reproduction. This compatibility level is similar to that of imidacloprid with *H. bacteriophora* and several other nematode species. Because compatibility levels to the same insecticide may differ among nematode species (Koppenhöfer and Grewal, 2005), compatibility of chlorantraniliprole with other nematodes species needs to be determined. The observed compatibility not only makes application of nematode-chlorantraniliprole combination easier but also facilitates their use in integrated pest management systems.

Similar to nematode-imidacloprid combinations *H. bacteriophora*-chlorantraniliprole combinations offer a less toxic alternative to presently used organophosphates and carbamates for curative white grub control. However, chlorantraniliprole has significantly lower toxicity and higher environmental/ecological safety even compared to imidacloprid (Lahm et al., 2007).

Our study was not designed to determine the economics of the examined combinations, and more field experiments with lower single agent rates are necessary to give a better idea about the economics. To be an economically viable option, combination treatments have to provide higher control of the white grubs than the better of the single agents at twice the rate used in the combination. Only in our first field experiment could we make such comparisons. In this experiment, the combination of *H. bacteriophora* (0.63×10^9 IJs/ha) and chlorantraniliprole (0.125 kg ai/ha) was not more effective against *A. orientalis* or *P. japonica* than the better of the two single agents at twice the rate (i.e., chlorantraniliprole at 0.25 kg ai/ha). Recently, however, Koppenhöfer and Fuzy (in press) showed that synergistic interactions also occur when *H. bacteriophora* and imidacloprid are applied against younger larvae (i.e., second instar and early third instar), and that similar control levels could be achieved with reduced nematode and imidacloprid rates. Chlorantraniliprole, like imidacloprid, tends to be more effective against younger larvae (Koppenhöfer, unpublished data). If rates of both nematode and chlorantraniliprole could be further reduced when applied against younger larvae combinations could become economically

viable. Further research should address this possibility along with the interaction of chlorantraniliprole with other entomopathogenic nematode species.

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