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Synergies between biological and neonicotinoid insecticides for the curative control of the white grubs *Amphimallon majale* and *Popillia japonica*

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ABSTRACT

Synergistic combinations of biological and chemical insecticides might yield promising alternatives for soil insect pest management. In turfgrass of the Northeast U.S., control of root-feeding scarab larvae is highly dependent on conventional insecticides. Studies on interactions between entomopathogenic nematodes and neonicotinoid insecticides, however, demonstrate the feasibility of synergies as an approach for reduced-risk curative control. To understand the breadth of potential synergies, we screened numerous combinations of biological control agents with sublethal doses of neonicotinoids against third instars. Interactions were characterized as synergistic, additive or antagonistic. The most promising combinations identified in laboratory bioassays were advanced to greenhouse pot studies and then to field trials featuring microplots with artificially infested populations. To reveal variation across scarab species, trials were conducted on *Amphimallon majale* and *Popillia japonica*. Synergies were consistent across trials and specific to white grub species. For *A. majale*, synergistic combinations of *Heterorhabditis bacteriophora* with imidacloprid and clothianidin were discernible in laboratory, greenhouse and field trials. For *P. japonica*, synergistic combinations of *Beauveria bassiana* and *Metarhizium anisopliae* with both neonicotinoids were discernible in the laboratory and greenhouse, but not in the field. For both species, antagonistic interactions were discernible between *Bt*-products and both neonicotinoids. While nematode-neonicotinoid synergies among scarab larvae have been examined before, fungi-neonicotinoid synergies are unreported. In the context of previous studies, however, no patterns emerge to explain variation across target species or control agent. Further study of non-additive interactions will guide how biological and chemical products could be combined to enhance soil insect pest management.

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1. Introduction

The tandem application of biological and chemical insecticides to achieve a greater total effect than the sum of their individual effects may be a promising approach for insect pest management in different agricultural systems. One example is a low rate of the insecticide imidacloprid in combination with the fungal entomopathogen *Beauveria bassiana* (Balsamo) Vuill., which exerts a synergistic effect on mortality and mycosis in the Colorado potato beetle (*Leptinotarsa decemlineata* Say) (Anderson et al., 1989; Furlong and Groden, 2001). Among soil insect pests, in particular, suppression can be enhanced when fungal and nematode pathogens are combined with neonicotinoid insecticides. Imidacloprid, for instance, was shown to be a synergist for the fungal entomopathogen *Metarhizium anisopliae* (Metchnikoff) Sorokin against the burrower bug *Cyrtomenus bergi* Froeschner (Jaramillo et al., 2005), as well as for *B. bassiana* against the root weevil *Diaprepes abbreviatus* (L.) (Quintela and McCoy,

1997, 1998). Imidacloprid and other neonicotinoids are also known synergists of entomopathogenic nematodes against scarab beetle larvae (Koppenhöfer and Kaya, 1998; Koppenhöfer et al., 2003). The strategic combination of biological controls with reduced rates of chemical insecticide synergists may therefore represent a valuable approach for the suppression of soil insect pests.

Root-feeding white grubs (Coleoptera: Scarabaeidae) are a major global pest of diverse agricultural crops (Jackson and Klein, 2006). Across extensive areas of the United States they are the most damaging group of soil insects in turfgrass, nurseries and ornamentals (Potter, 1998; Vittum et al., 1999). While a complex of at least eight species is problematic in turfgrass of the Northeast United States, the most challenging are four exotic species introduced in the early 1900's. These include the Japanese beetle (*Popillia japonica* Newman), the oriental beetle [*Anomala orientalis* (Waterhouse)], the Asiatic garden beetle [*Maladera castanea* (Arrow)] and the European chafer [*Amphimallon majale* (Razoumowsky)] (Alm et al., 1999; Potter and Held, 2002; Bughrara et al., 2003; Koppenhöfer and Fuzy, 2003a).

All four exotic species have a 1-yr life cycle in the Northeast U.S. (Potter, 1998; Vittum et al., 1999). Adults emerge in late spring

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(*A. majale* and *M. castanea*) or summer (*A. orientalis* and *P. japonica*) to lay eggs in the soil. First instars develop over 4–6 wk, when they can be effectively targeted for suppression with preventive insecticides, such as imidacloprid. Larvae of all four species are third instar by the end of the summer; at this point suppression shifts to faster acting curative insecticides, such as the organophosphate trichlorfon. Third instars feed voraciously on roots which can lead to extensive turf loss. Additional indirect damage can be experienced due to the activity of mammalian predators digging for grubs, even in areas where direct damage is not visible. After overwintering, third instars emerge in early spring to feed for ≈ 2 wk before pupation.

Pest management practitioners in this system would benefit from curative and biologically-based control alternatives for white grub suppression. In contrast to early season preventive applications at the time of egg hatch, decisions to make late season curative applications could consider action thresholds as the larvae are large enough to sample through scouting. In contrast to conventional insecticides, biological alternatives would likely harbor fewer non-target effects and offer options for plant protection in municipalities, counties or states under more restrictive pesticide use legislation. Reliance on chemical insecticides persists, in part, because biologically-based commercial products are few, unreliable and limited. For instance, a commercial formulation of the bacteria that causes “milky disease”, *Paenibacillus popilliae* Dutky, is only formulated for one species (*P. japonica*), has a slow effect, and is unproven in field trials (Redmond and Potter, 1995). Entomopathogenic nematodes of the genera *Heterorhabditis* and *Steinernema* are commercially available, but their field efficacy is too variable owing to inconsistency and unreliability of the formulation and quality of the final product (Grewal and Peters, 2005). The first commercial formulation of *M. anisopliae* (strain F52) in the United States was recently approved (USEPA, 2003), but even though turf-infesting white grubs are on the label, the product has not been rigorously field-tested.

Biological–chemical synergisms are one approach that might be exploited for reduced-risk white grub management in turf. This is substantiated by a series of studies revealing how third instars are more susceptible to entomopathogenic nematodes when challenged by other antagonists such as endophytic host plants (Grewal et al., 1995) and reduced rates of neonicotinoids. With respect to neonicotinoids, the first specific evidence was reported by Koppenhöfer and Kaya (1998) on synergistic interactions between *H. bacteriophora* and imidacloprid against *Cyclocephala hirta* (LeConte) and *C. pasadenae* Casey. Further studies identified other neonicotinoid synergists for nematodes (i.e., acetamiprid and thiamethoxam) and confirmed the susceptibility of other white grub species (i.e., *A. majale*, *M. castanea*) to those synergies (Koppenhöfer et al., 2000a, 2002; Koppenhöfer and Fuzy, 2003b). While the mechanisms involved in the interaction between neonicotinoids and nematodes are not fully understood, among white grubs it is likely that disruption of the insect’s grooming behavior facilitates host attachment by infective juvenile nematodes (Koppenhöfer et al., 2000b).

Beyond the aforementioned antagonists, little is known about the potential of other non-chemical products to combine with neonicotinoids and yield synergies that could be exploited for insect pest management. Our goals were to broaden this understanding and to reveal opportunities for curative grub control that featured biologicals in tandem with reduced rates of chemical insecticides. Because of the ability of neonicotinoids to modify white grub defensive and grooming behavior, we hypothesized that exposure to sublethal doses would increase vulnerability to certain biological agents. Spot treatments of neonicotinoids at reduced rates should lead to environmental benefits by reducing non-target impacts (Peck, 2009) and environmental contamination

(Gilrein and Yeh, 2004). Our approach was to screen numerous combinations under controlled laboratory conditions against third instars. Beyond being the target of curative application, this developmental stage was chosen because it is the most injurious, the most difficult to control, and the easiest to manipulate. The interactions were characterized as synergistic, additive or antagonistic. The most promising combinations (based on synergy and efficacy) were advanced to greenhouse pot studies, and then to field studies featuring microplots with artificially infested populations. To understand how synergistic combinations might vary across grub species, trials were conducted on two of the dominant species in the Northeast United States, *P. japonica* and *A. majale*.

2. Methodology

2.1. Source of insects

Third instars collected from the field were used to conduct all laboratory, greenhouse and field experiments. *Popillia japonica* was collected on 29 November 2005 in Fulton, NY (Battle Island Golf Course, Oswego Co.), on 7–8 November 2006 in Geneva, NY (Seneca Lake Country Club, Ontario Co.) and on 6–7 November 2007 in Victor, NY (Parkview Fairways Golf Course, Ontario Co.). *Amphimallon majale* was collected on 15–16 November 2005 and on 31 October 2006 in Lake George, NY (Queensbury Country Club, Warren Co.) and on 6–7 November 2007 in Victor, NY (Parkview Fairways Golf Course, Ontario Co.). Larvae were maintained in wooden boxes with soil and a piece of sod as a food source from the same sites of collection. Boxes were held in a walk-in cooler at 10 °C until the start of trials.

2.2. Treatments

Biological insecticides were applied at recommended rates in combination with two chemical insecticides applied at 1/2 and 1/4 the highest label rate of 0.45 kg AI/ha. The chemical insecticides were clothianidin (Arena 50 WDG; Valent, Walnut Creek, CA) and imidacloprid (Merit 75 WP; Bayer, Durham, NC). The biologicals included products based on entomopathogenic fungi, nematodes and bacteria.

The fungal entomopathogens were represented by one strain of *B. bassiana* (Botanigard ES; Emerald BioAgriculture, Okemos, MI) (8.14×10^{15} conidia/ha) and two strains of *M. anisopliae* (Met F52; Novozymes Biologicals, Salem, VA and Met NYSAES) (7.94×10^{15} conidia/ha). The NYSAES strain was isolated from *P. japonica* collected in Fulton, NY (Battle Island Golf Course, Oswego Co.) and was propagated on sterile Sabouraud Dextrose Agar (10 g peptone, 40 g dextrose, 15 g agar, 1 L distilled water) plus 1% yeast (10 g yeast extract). After inoculation with conidia, Petri dishes were maintained for 18–20 d in a growth chamber at 27 °C and photoperiod of 12:12 h light:dark. For the application, conidia were scraped from the plate into a 1-L flask containing 500 ml of sterilized distilled water with 0.05% Tween 80 (Fisher Scientific, Pittsburgh, PA). The conidial concentration was determined with a hemacytometer (American Optical, Buffalo, NY) and adjusted to the required concentration.

The entomopathogenic nematodes were represented by one strain of *H. bacteriophora* (Heteromask; BioLogic, Willow Hill, PA) (2.04×10^9 IJ/ha) and two strains of *Heterorhabditis* sp. (NYSAES Nema 1 and NYSAES Nema 2) (2.04×10^9 IJ/ha). NYSAES Nema 1 was isolated from soil samples taken in Fulton, NY and NYSAES Nema 2 was isolated from a third instar *A. majale* collected in Saratoga Springs, NY (Saratoga Spa Golf, Saratoga Co.). Both strains were cultured in the last instar of the greater wax moth, *Galleria mellonella* L. The emerging infective juveniles (IJ) were harvested

from white traps the same day of application (Kaya and Stock, 1997).

The bacterial products were represented by *P. popilliae* (Milky Spore; St. Gabriel Laboratories, Orange, VA) (2.5 kg AI/ha) and *Bacillus thuringiensis*. The *Bt*-products were selected based on availability. *Bt* var. *galleriae* was an experimental formulation (650 g AI/ha) with activity against lepidopteran larvae and *Bt* var. *tenebrionis* (Novodor FC; Valent BioSciences, Libertyville, IL) (935 ml AI/ha) was a commercial product.

2.3. Laboratory experiments

For the laboratory experiments, insects were maintained in a walk-in environmental chamber under controlled climate conditions (complete darkness, 90–95% RH, 25 °C) at the NYSAES, Geneva, NY. Assay units were 30-ml plastic cups filled with 30 g of screened soil raised to 10.0% (w/w) moisture. The soil was sandy loam (83.0% sand, 12.0% silt, 5.0% clay) with 0.79% organic matter and pH 7.09. A pinch of grass seed [Shady-Green Lawn Mixture: *Festuca rubra* L. (43.9%), *Lolium perenne* L. (44%), and *Poa pratensis* L. (9.8%); Agway, Richmond, VA] was added as a food source 24 h before infestation. After being held at room temperature for 24 h, one larva was released into each cup and replaced if it did not burrow into the soil within 3 h. The treatment applications consisted of the full rate of the biological alone, 1/2 or 1/4 rate of the neonicotinoid alone, and the combination of biological and neonicotinoid. Treatments were applied in 2 ml of water total: 1 ml each for the biological and chemical insecticides. When only one control agent was applied, 1 ml of water was added. Untreated checks received 2 ml of water.

Each assay had six repetitions of 10 cups and 10 grubs, and was conducted once each year. Studies in year 1 were initiated December 20 (*P. japonica*) and December 22 (*A. majale*) 2005. Studies in year 2 were initiated December 15 (*P. japonica*) and 18 December (*A. majale*) 2006. In year 2, neither *Heterorhabditis* sp. strain Nema 1 nor Nema 2 was available for application. Evaluations were made at 10, 20 and 30 d after treatment (DAT) to measure mortality rates. To do this, the contents of each cup were emptied onto a piece of paper, the status of the grub was assessed (dead or alive), and the contents were replaced in the cup with a pinch of additional grass seed. These protocols were modified from Morales-Rodriguez et al. (accepted for publication).

2.4. Greenhouse experiments

Greenhouse experiments were also conducted at the NYSAES. The average greenhouse temperature was 14.0 °C (8.5–27.5 °C) and the photoperiod was 14:10 h light:dark. Assay units were 1-L pots filled with soil and seeded with *L. perenne* (Tri-Rye; Agway, Richmond, VA). The potting soil was sandy loam (84.0% sand, 11.0% silt, 5.0% clay) with 8.43% organic matter and pH 6.85. Grass was maintained for 10–12 wk, watered every 2 d, and cut to a height of 5.0 cm and fertilized (20-20-20; Scotts Miracle-Gro Products, Marysville, OH) weekly.

Five larvae were released into each pot and those that did not burrow into the soil within 24 h were replaced. Treatments were applied in 100 ml of water: 50 ml each for the biological and chemical insecticides. When only one control agent was applied, 50 ml of water was added. Untreated checks received 100 ml of water. Each assay had 10 pots (repetitions) and was conducted once each year. Studies in year 1 were initiated December 20 (*P. japonica*) and December 22 (*A. majale*) 2006. Studies in year 2 were initiated January 15 (*P. japonica*) and January 29 (*A. majale*) 2008.

Treatments for each grub species were selected based on those that exhibited a synergistic interaction in laboratory assays. The *P. japonica* treatments were *B. bassiana* and clothianidin

at 1/2 and 1/4 label rate alone and in combination; Met 52 and clothianidin at 1/2 label rate alone and in combination; and Met NYSAES and imidacloprid at 1/2 label rate alone and in combination. The *A. majale* treatments were *H. bacteriophora* and clothianidin and imidacloprid at 1/2 and 1/4 label rate alone and in combination. After applications, all pots were arranged in a completely randomized design. Destructive evaluations were made at 30 DAT to measure mortality rates.

2.5. Field experiments

Field experiments were conducted in microplots with artificially infested populations at sites that had not been treated with insecticides within 5 yr. Studies with *P. japonica* were conducted on irrigated turf at the Turf and Landscape Research Center, Cornell University, Ithaca, NY. Mowing height was 6.5 cm, thatch depth was 0.2–0.5 cm and turf composition was *Lolium* spp. (35%), *Poa annua* L. (19%), *Festuca* spp. (2%) and broad-leaf weeds (44%). Soil was sandy loam (63.0% sand, 26.0% silt, 9.0% clay) with 2.6% organic matter and pH 5.8. Previous to the start of the experiment, natural populations of white grubs were detected at a density of 30–40 grubs/m², with a species composition of 90–95% *P. japonica* and 5–10% *A. majale*.

Experiments with *A. majale* were conducted in experimental turf plots at the NYSAES. Mowing height was 8.5 cm, thatch depth was 0.4–0.6 cm and turf composition was *Lolium* spp. (18.8%), *Digitaria* spp. (75.0%) and broad-leaf weeds (6.2%). Soil was silty clay loam (12.5% sand, 55.0% silt, 32.5% clay) with 5.7% organic matter and pH 6.5. Previous to the start of the experiment, a low natural population of *P. japonica* was detected at a density of ≤5 grubs/m².

The microplots were PVC rings (30.5 cm diameter, 7.6 cm height) that were pushed completely into the soil 1 wk before infestation. Fifteen *P. japonica* or 10 *A. majale* third instars were released into each ring 24 h before treatment application. Larvae that did not burrow into the soil within 3 h were replaced. There were ten replicated microplots per treatment and these were arranged in a randomized block design. The *P. japonica* treatments were applied on 29 October 2007 (air temperature 5.5 °C; soil temperature 10.7 °C at 2.5 cm depth and 10.9 °C at 7.6 cm depth; skies sunny). The *A. majale* treatments were applied on 26 October 2007 (air temperature 13.0 °C; soil temperature 11.9 °C at 2.5 cm depth and 12.2 °C at 7.6 cm depth; skies partly cloudy). The treatments were the same as those applied in the greenhouse for each species. Applications were made in 500 ml of water and the untreated check received only water. All applications were made using a watering can followed by 0.95 cm of irrigation. Destructive evaluations were made in five microplots at 30 DAT (late fall) and five at 174 DAT (early spring) to measure mortality rates.

2.6. Statistics

In the laboratory and greenhouse experiments, percent mortality was corrected for mortality in the untreated check (Abbott, 1925). In the field experiments, mortality was corrected for the average number of larvae recovered from the untreated check. Percent mortality data were normalized using an arcsine square root transformation and subjected to analysis of variance (ANOVA). Synergistic, additive and antagonistic interactions between agents in the combination treatments were determined using a X^2 test (Benz, 1971; MacVay et al., 1977; Koppenhöfer and Kaya, 1998; Koppenhöfer and Fuzy, 2003b). The expected interaction mortality value, M_E , for combined agents was calculated using the formula $M_E = M_B + M_N (1 - M_B/100)$, where M_B and M_N are the observed percent mortalities caused by the biological and neonicotinoid products alone, respectively. Results from the X^2 tests were compared to the X^2 table value for 1 df, using the formula

$X^2 = (M_{BN} - M_E)^2 / M_E$, where M_{BN} is the observed mortality for the biological-neonicotinoid combinations. A non-additive effect between the two agents was suspected when the X^2 value exceeded the table value (Koppenhöfer and Fuzy, 2003b). If the difference $M_{BN} - M_E$ had a positive or negative value, a significant interaction was then considered synergistic or antagonistic, respectively. Data from the field experiments were additionally assessed for an effect of overwintering. To do that each treatment mean was tested individually in a contrast between 30 and 174 DAT evaluations. Differences among means were considered significant at $P < 0.05$. All statistical analyses were performed using SAS (SAS Institute, 2002).

3. Results

3.1. Laboratory experiments

The range of laboratory mortality in the untreated check was 5–15% for both species. Because there was an effect of year ($F = 10.2$; $df = 1, 714$; $P = 0.002$) on mortality, each year was analyzed separately. In 2005, there was a significant effect of treatment ($F = 27.9$; $df = 49, 500$; $P < 0.001$), white grub species ($F = 1440.5$; $df = 1, 500$; $P < 0.001$) and their interaction ($F = 6.7$; $df = 50, 500$; $P < 0.001$) on mortality at 30 DAT. This result was consistent in 2006 for treatment ($F = 13.1$; $df = 49, 500$; $P < 0.001$), white grub species ($F = 649.7$; $df = 49, 500$; $P < 0.001$) and their interaction ($F = 6.5$; $df = 101, 714$; $P < 0.001$). Overall, for the fungi-neonicotinoid combinations, *P. japonica* was significantly more susceptible than *A. majale* (Tables 1–4). Of the 24 combinations evaluated over both years, 11 were synergistic for *P. japonica* but none for *A. majale* (Tables 1–4). For the nematode-neonicotinoid combinations, in contrast, *A. majale* was significantly more susceptible than *P. japonica*. Of the 16 combinations with *H. bacteriophora*, 7 were synergistic for *A. majale* and only 1 for *P. japonica* (Tables 1–4). For the bacteria-neonicotinoid combinations, only antagonistic or additive effects were detected. Of the 24 combinations, 8 were antagonistic for *A. majale* and 11 for *P. japonica* (Tables 1–4).

For *P. japonica* and *B. bassiana*, a synergistic interaction was detected for clothianidin 1/2 in both years (Tables 1 and 2). There was an additional synergy for imidacloprid 1/2 and clothianidin 1/4 in 2005 and imidacloprid 1/4 in 2006. For Met F52, a synergistic interaction was detected for clothianidin 1/2 in both years. There was an additional synergy for imidacloprid 1/2 in 2005. For Met NYSAES, a synergistic interaction was detected for imidacloprid 1/2 in both years. There was an additional synergy for clothianidin 1/2 in 2005. An antagonistic interaction was detected for clothianidin 1/4 in 2006. For *A. majale* and *B. bassiana*, an antagonistic interaction was detected for imidacloprid 1/4 and clothianidin 1/4 in 2006 (Tables 3 and 4). For Met F52, an antagonistic interaction was detected with clothianidin 1/4 and 1/2 and imidacloprid 1/2 in 2006. For Met NYSAES, an antagonistic interaction was detected for clothianidin 1/2 in 2006. For both white grub species, all other interactions between fungal entomopathogens and neonicotinoids were additive.

For *P. japonica* and NYSAES Nema 1, a synergistic interaction was detected for clothianidin 1/2 in 2005 (Table 1). For *A. majale* and *H. bacteriophora*, a synergistic interaction was detected for imidacloprid 1/2 and 1/4 and clothianidin 1/2 and 1/4 in both years, with the exception of clothianidin 1/4 in 2006 which was only additive (Tables 3 and 4). For both white grub species, all other interactions between entomopathogenic nematodes and neonicotinoids were additive.

For *P. japonica* and *P. popilliae*, an antagonistic interaction was detected for imidacloprid 1/2 and 1/4 in 2005 and clothianidin 1/2 in 2006 (Tables 1 and 2). For *Bt* var *galleriae*, an antagonistic interaction was detected for imidacloprid 1/2 and 1/4 in 2005 and clothianidin

1/2 in 2006. For *Bt* var *tenebrionis*, an antagonistic interaction was detected for imidacloprid 1/2 and 1/4 in 2005 and imidacloprid 1/2 and 1/4 and clothianidin 1/2 and 1/4 in 2006. For *P. popilliae* and *A. majale*, an antagonistic interaction was detected for imidacloprid 1/2 and 1/4 in 2005 and clothianidin 1/2 in 2006 (Tables 3 and 4). For *Bt* var *galleriae*, an antagonistic interaction was detected for imidacloprid 1/2 in 2005 and clothianidin 1/2 in 2006. For *Bt* var *tenebrionis*, an antagonistic interaction was detected for imidacloprid 1/4 in both years. There was an antagonism for clothianidin 1/4 in 2006. For both white grub species, all other interactions between bacterial products and neonicotinoids were additive.

Among all treatments that showed synergistic interactions against *P. japonica* at 30 DAT in 2005, only *B. bassiana* in combination with clothianidin 1/2 and 1/4 expressed the effect at 10 and 20 DAT (Fig. 1). Neither Met NYSAES in combination with imidacloprid 1/2 nor Met F52 in combination with clothianidin 1/2 expressed the effect earlier than 30 DAT. In 2006, none of the synergies was expressed before 30 DAT. For *A. majale*, all treatments that showed synergistic interaction effects were expressed as early as 10 and 20 DAT in 2005 (Fig. 2). In 2006, none of the synergies was expressed before 30 DAT.

3.2. Greenhouse experiments

The range of greenhouse mortality in the untreated check was 4–18% for both species. In general, treatment mortalities declined in the greenhouse with respect to the laboratory. For *P. japonica*, mortality due to *B. bassiana* in combination with clothianidin 1/2 declined from 83.3 (year 1) and 63.3% (year 2) in the laboratory, to 29.5 (year 1) and 53.0% (year 2) in the greenhouse (Tables 3–5). For Met NYSAES in combination with clothianidin 1/2, mortalities declined from 83.3 and 41.7% to 18.0 and 34.0%. A synergistic interaction was maintained in both years for *B. bassiana* in combination with clothianidin 1/2, and for Met F52 in combination with clothianidin 1/2 (Table 5). All other interactions were additive for *P. popilliae*.

For *A. majale*, mortality due to *H. bacteriophora* in combination with clothianidin 1/2 declined from 58.3 (year 1) and 58.3% (year 2) in the laboratory to 32.0 (year 1) and 36.0% (year 2) in the greenhouse (Table 5). In combination with clothianidin 1/4, mortalities declined from 41.7% and 51.7% to 28.0% and 18.0%. For *H. bacteriophora* in combination with imidacloprid 1/2, mortalities declined from 75.0% and 48.3% to 40.0% and 42.0%. In combination with imidacloprid 1/4, mortalities declined from 80.0% and 58.3% to 51.0% and 58.0%. A synergistic interaction was maintained in both years for *H. bacteriophora* in combination with clothianidin 1/2 and with imidacloprid 1/2 and 1/4. All other interactions were additive for *A. majale* (Table 5).

3.3. Field experiments

The average number of *P. japonica* recovered in the untreated check was 5.2 at 30 DAT, or 34.7% of the initial infestation, and 10.2 at 174 DAT, or 67.8% of the initial infestation. Average recovery of *A. majale* was 9.1 at 30 DAT, or 90.1% of the initial infestation, and 6.9 at 174 DAT, or 69.3% of the initial infestation.

For *P. japonica*, no mortality was detected at 30 DAT for any of the treatments (Table 6). At 174 DAT an antagonistic interaction was detected for *B. bassiana* in combination with clothianidin 1/2. All other interactions were additive. Overwintering had a significant effect on mortality for all treatments. Mortality was significantly higher at 174 versus 30 DAT for *B. bassiana* in combination with clothianidin 1/2 ($F = 17.6$; $df = 1, 32$; $P < 0.001$) and clothianidin 1/4 ($F = 23.0$; $df = 1, 32$; $P < 0.001$), for Met F52 in combination with imidacloprid 1/2 ($F = 17.6$; $df = 1, 32$; $P < 0.001$), and for Met NYSAES in combination with clothianidin 1/2 ($F = 29.1$; $df = 1, 32$; $P < 0.001$).

Table 1Laboratory mortality (mean \pm SE) of third instar *Popillia japonica* and the interaction among different combinations of biological and neonicotinoid insecticides at 30 DAT in 2005.

Treatment	Measurement ^a	Rate 1/2 ^b			Rate 1/4 ^c		
		Mortality	χ^2	Effect	Mortality	χ^2	Effect
<i>B. bassiana</i> GHA							
Imidacloprid	Observed	75.0 \pm 5.0	7.76	Synergistic	53.3 \pm 5.6	0.27	Additive
	Expected	58.3 \pm 6.3			56.7 \pm 8.7		
Clothianidin	Observed	83.3 \pm 4.9	70.48	Synergistic	80.0 \pm 8.6	23.80	Synergistic
	Expected	36.7 \pm 8.6			50.0 \pm 8.3		
<i>M. anisopliae</i> Met F52							
Imidacloprid	Observed	78.3 \pm 4.7	46.17	Synergistic	61.7 \pm 7.5	0.81	Additive
	Expected	40.0 \pm 5.1			53.3 \pm 5.6		
Clothianidin	Observed	78.3 \pm 4.8	46.17	Synergistic	61.7 \pm 7.0	3.27	Additive
	Expected	61.7 \pm 9.3			60.0 \pm 7.2		
<i>M. anisopliae</i> NYSAES							
Imidacloprid	Observed	83.3 \pm 4.9	9.33	Synergistic	45.0 \pm 9.9	3.05	Additive
	Expected	68.3 \pm 7.7			66.7 \pm 6.7		
Clothianidin	Observed	66.7 \pm 9.2	41.67	Synergistic	56.7 \pm 9.5	0.29	Additive
	Expected	46.7 \pm 5.4			60.0 \pm 7.1		
<i>H. bacteriophora</i>							
Imidacloprid	Observed	100.0 \pm 0.0	0.0	Additive	100.0 \pm 0.0	0.0	Additive
	Expected	100.0 \pm 0.0			100.0 \pm 0.0		
Clothianidin	Observed	100.0 \pm 0.0	0.0	Additive	100.0 \pm 0.0	0.0	Additive
	Expected	100.0 \pm 0.0			100.0 \pm 0.0		
<i>Heterorhabditis</i> sp. NYSAES Nema 1							
Imidacloprid	Observed	58.3 \pm 5.4	0.06	Additive	53.3 \pm 4.9	0.05	Additive
	Expected	61.7 \pm 6.2			60.0 \pm 5.8		
Clothianidin	Observed	63.3 \pm 7.1	18.74	Synergistic	53.3 \pm 6.1	0.38	Additive
	Expected	40.0 \pm 5.6			53.3 \pm 5.1		
<i>Heterorhabditis</i> sp. NYSAES Nema 2							
Imidacloprid	Observed	55.5 \pm 5.7	1.13	Additive	53.3 \pm 4.2	0.19	Additive
	Expected	50.0 \pm 4.9			63.3 \pm 6.1		
Clothianidin	Observed	48.3 \pm 10.1	2.06	Additive	60.0 \pm 5.7	1.63	Additive
	Expected	41.3 \pm 8.7			56.7 \pm 4.9		
<i>P. popilliae</i>							
Imidacloprid	Observed	26.7 \pm 7.1	24.26	Antagonistic	50.0 \pm 7.3	3.80	Additive
	Expected	83.3 \pm 5.6			81.7 \pm 9.2		
Clothianidin	Observed	38.3 \pm 8.7	3.67	Additive	53.3 \pm 4.2	1.03	Additive
	Expected	61.6 \pm 7.3			75.0 \pm 6.8		
<i>Bt</i> var. <i>galleriae</i>							
Imidacloprid	Observed	26.7 \pm 6.2	16.38	Antagonistic	38.3 \pm 5.4	5.49	Antagonistic
	Expected	53.3 \pm 4.7			51.7 \pm 8.1		
Clothianidin	Observed	46.7 \pm 6.1	1.89	Additive	48.3 \pm 6.0	0.05	Additive
	Expected	41.7 \pm 5.1			45.0 \pm 5.2		
<i>Bt</i> var. <i>tenebrionis</i>							
Imidacloprid	Observed	33.3 \pm 8.4	13.35	Antagonistic	59.4 \pm 9.5	3.94	Antagonistic
	Expected	73.3 \pm 9.4			100.0 \pm 0.0		
Clothianidin	Observed	48.3 \pm 13.2	0.22	Additive	48.3 \pm 4.8	0.95	Additive
	Expected	51.7 \pm 9.3			65.0 \pm 5.7		

^a Observed = efficacy of both control agents applied at the same time. Expected = sum of efficacy of each control agent applied separately.^b 1/2 = (0.225 kg AI/ha) half of the high label rate recommendation for white grub control.^c 1/4 = (0.123 kg AI/ha) quarter of the high label rate recommendation for white grub control.

For *A. majale*, all combinations were additive at 30 DAT but a synergistic interaction was detected for *H. bacteriophora* in combination with clothianidin 1/2 and imidacloprid 1/2 and 1/4 at 174 DAT (Table 6). The combination with clothianidin 1/4 was additive. Overwintering had a significant effect on mortality for only one combination. Mortality was significantly higher at 174 DAT for *H. bacteriophora* in combination with imidacloprid 1/4 ($F = 3.15$; $df = 1, 32$; $P = 0.005$). No overwintering effect was detected for *H. bacteriophora* in combination with imidacloprid 1/2 ($F = 3.43$; $df = 1, 32$; $P = 0.072$), clothianidin 1/4 ($F = 3.89$; $df = 1, 32$; $P = 0.057$) or clothianidin 1/2 ($F = 3.25$; $df = 1, 32$; $P = 0.081$).

4. Discussion

Among the biological-neonicotinoid combinations tested here, synergistic interactions were relatively uncommon and involved

only entomopathogenic nematodes and fungi, not bacteria. Of the 80 experimental combinations evaluated, interactions were synergistic/additive/antagonistic 15/52/13 times for *P. japonica* and 17/49/14 times for *A. majale*. Moreover, synergies were remarkably consistent across trials, were specific to white grub species, and diminished in strength from lab to greenhouse to field. The most promising synergistic combinations for *P. japonica* were the entomopathogenic fungi *B. bassiana* and *M. anisopliae* Met F52 with clothianidin, and *M. anisopliae* NYSAES with imidacloprid. This was discernible in each of the two laboratory trials; some combinations persisted into the greenhouse but none persisted into the field. For *A. majale*, the most promising synergistic combinations were between the entomopathogenic nematode *H. bacteriophora* and both neonicotinoids; those results were discernible in all laboratory and greenhouse trials and into the field. Finally, antagonistic interactions between *Bt*-products and both neonicotinoids were common to both white grub species.

Table 2
Laboratory mortality (mean \pm SE) of third instar *Popillia japonica* and the interaction among different combinations of biological and neonicotinoid insecticides at 30 DAT in 2006.

Treatment	Measurement ^a	Rate 1/2 ^b			Rate 1/4 ^c			
		Mortality	χ^2	Effect	Mortality	χ^2	Effect	
<i>B. bassiana</i> GHA	Imidacloprid	Observed	38.3 \pm 9.5	1.39	Additive	43.3 \pm 2.1	3.92	Synergistic
		Expected	41.7 \pm 5.4			35.0 \pm 8.5		
	Clothianidin	Observed	63.3 \pm 2.1	15.78	Synergistic	45.0 \pm 4.3	2.23	Additive
		Expected	43.3 \pm 7.6			40.0 \pm 7.7		
<i>M. anisopliae</i> Met F52	Imidacloprid	Observed	26.7 \pm 3.3	1.65	Additive	23.3 \pm 3.3	1.80	Additive
		Expected	21.7 \pm 3.1			18.3 \pm 3.1		
	Clothianidin	Observed	58.3 \pm 4.8	41.68	Synergistic	28.3 \pm 4.8	1.54	Additive
		Expected	26.7 \pm 4.2			23.3 \pm 2.1		
<i>M. anisopliae</i> NYSAES	Imidacloprid	Observed	55.0 \pm 2.2	8.16	Synergistic	41.7 \pm 6.0	1.27	Additive
		Expected	41.7 \pm 5.4			38.3 \pm 4.0		
	Clothianidin	Observed	41.7 \pm 10.8	0.01	Additive	25.0 \pm 6.2	4.87	Antagonistic
		Expected	46.7 \pm 9.3			43.3 \pm 4.9		
<i>H. bacteriophora</i>	Imidacloprid	Observed	33.3 \pm 4.2	0.59	Additive	23.3 \pm 5.6	0.35	Additive
		Expected	31.7 \pm 5.4			28.3 \pm 3.1		
	Clothianidin	Observed	28.3 \pm 4.0	0.78	Additive	30.0 \pm 3.7	0.01	Additive
		Expected	36.7 \pm 4.2			33.3 \pm 4.9		
<i>P. popilliae</i>	Imidacloprid	Observed	16.7 \pm 3.3	1.38	Additive	18.3 \pm 3.1	1.38	Additive
		Expected	23.3 \pm 3.3			20.0 \pm 4.5		
	Clothianidin	Observed	13.3 \pm 2.1	6.88	Antagonistic	15.0 \pm 2.2	3.26	Additive
		Expected	28.3 \pm 4.0			25.0 \pm 2.2		
<i>Bt</i> var. <i>galleriae</i>	Imidacloprid	Observed	20.0 \pm 2.6	2.88	Additive	23.3 \pm 6.1	0.35	Additive
		Expected	31.7 \pm 4.0			28.3 \pm 1.7		
	Clothianidin	Observed	10.0 \pm 2.6	16.41	Antagonistic	23.3 \pm 4.9	1.73	Additive
		Expected	36.7 \pm 4.2			33.3 \pm 3.3		
<i>Bt</i> var. <i>tenebrionis</i>	Imidacloprid	Observed	20.0 \pm 5.2	11.27	Antagonistic	26.7 \pm 3.3	4.06	Antagonistic
		Expected	46.7 \pm 6.1			43.3 \pm 3.3		
	Clothianidin	Observed	23.3 \pm 4.9	10.59	Antagonistic	26.7 \pm 4.2	6.08	Antagonistic
		Expected	51.7 \pm 6.0			48.3 \pm 4.8		

^a Observed = efficacy of both control agents applied at the same time. Expected = sum of efficacy of each control agent applied separately.

^b 1/2 = (0.225 kg AI/ha) half of the high label rate recommendation for white grub control.

^c 1/4 = (0.123 kg AI/ha) quarter of the high label rate recommendation for white grub control.

Further study of these non-additive interactions might shed light on how biological and chemical products could be combined to offer enhanced control of soil insect pests.

4.1. Specificity of synergies to grub species

The effects of the same biological-neonicotinoid combinations varied between white grub species. While the interactions between *H. bacteriophora* and both neonicotinoids were synergistic in *A. majale*, they were only additive in *P. japonica*. In contrast, interactions between *B. bassiana* and *M. anisopliae* and both neonicotinoids were synergistic in *P. japonica*, but only additive in *A. majale*. As demonstrated by Morales-Rodriguez et al. (accepted for publication) under the same experimental conditions, there is meaningful variation among white grub species in their susceptibility to individual control products. Among the four grub species tested in that study, *P. japonica* was the most susceptible to fungal entomopathogens (*B. bassiana* and *M. anisopliae*) while *A. majale* was the most susceptible to entomopathogenic nematodes (*H. bacteriophora*). Differences in relative susceptibility to biologicals applied individually are thereby consistent with the characterization of those biologicals as synergistic when combined with neonicotinoid insecticides.

With respect to nematodes, synergies were only detected for *A. majale*, and these were specifically between *H. bacteriophora* and imidacloprid. Clothianidin and *Heterorhabditis* sp. Nema 1 and Nema 2 were not involved in synergies. No patterns have emerged

from previous studies on how synergistic interactions vary across grub species and across neonicotinoid chemistry. Contrary to our results, Koppenhöfer et al. (2002) discerned a synergy between *H. bacteriophora* and imidacloprid for *P. japonica*, but not for *A. majale*. And while the same study showed a similar synergy in *P. japonica* with thiamethoxam, a later study only reported an additive effect for both imidacloprid and thiamethoxam (Koppenhöfer et al., 2003). In another study (Koppenhöfer et al., 2002), *H. bacteriophora* combined with imidacloprid and acetamiprid had only an additive effect on *A. majale* and *M. castanea*, but with thiamethoxam it had an antagonistic effect. There was, moreover, a synergistic effect for all combinations in *A. orientalis*.

With respect to fungi, synergies were only detected for *P. japonica*, and these were relatively broad-based, including *B. bassiana* with clothianidin (1/2 and 1/4) and imidacloprid (1/2), as well as both strains of *M. anisopliae* in combination with both neonicotinoids (1/2). Although we know of no other reports of synergistic interactions between fungi and neonicotinoids for white grubs, there are reports from other soil insects. Two examples based on low doses of imidacloprid are *M. anisopliae* under laboratory and greenhouse conditions against nymphs of *C. bergi* (Jaramillo et al., 2005), and both *B. bassiana* and *M. anisopliae* against larvae of *D. abbreviatus* (Quintela and McCoy, 1998).

Beyond target species and neonicotinoid chemistry, the specific strain of pathogen and the insecticide application rate may affect the type of interaction and the strength of synergistic effects. Polav-

Table 3

Laboratory mortality (mean \pm SE) of third instar *Amphimallon majale* and the interaction among different combinations of biological and neonicotinoid insecticides at 30 DAT in 2005.

Treatment	Measurement ^a	Rate 1/2 ^b			Rate 1/4 ^c		
		Mortality	χ^2	Effect	Mortality	χ^2	Effect
<i>B. bassiana</i> GHA							
Imidacloprid	Expected	10.0 \pm 4.5	0.0	Additive	6.7 \pm 3.3	1.66	Additive
	Observed	10.0 \pm 4.4			10.0 \pm 2.6		
Clothianidin	Expected	6.7 \pm 4.2	1.66	Additive	16.7 \pm 8.2	1.21	Additive
	Observed	10.0 \pm 3.7			6.7 \pm 2.1		
<i>M. anisopliae</i> Met F52							
Imidacloprid	Expected	10.0 \pm 4.5	1.11	Additive	6.7 \pm 3.3	3.75	Additive
	Observed	6.7 \pm 3.3			11.7 \pm 1.6		
Clothianidin	Expected	6.7 \pm 4.2	0.41	Additive	6.6 \pm 4.3	2.72	Additive
	Observed	5.0 \pm 2.2			1.7 \pm 1.7		
<i>M. anisopliae</i> NYSAES							
Imidacloprid	Expected	15.0 \pm 5.0	3.38	Additive	11.7 \pm 5.4	3.62	Additive
	Observed	23.3 \pm 6.1			20.0 \pm 5.2		
Clothianidin	Expected	13.3 \pm 5.5	0.15	Additive	20.0 \pm 5.8	2.88	Additive
	Observed	10.0 \pm 3.7			11.7 \pm 4.0		
<i>H. bacteriophora</i>							
Imidacloprid	Expected	33.3 \pm 9.5	62.45	Synergistic	30.0 \pm 7.7	93.44	Synergistic
	Observed	75.0 \pm 6.7			80.0 \pm 3.7		
Clothianidin	Expected	28.3 \pm 9.1	31.41	Synergistic	23.3 \pm 6.6	62.45	Synergistic
	Observed	58.3 \pm 6.0			41.7 \pm 7.5		
<i>Heterorhabditis</i> sp. NYSAES Nema 1							
Imidacloprid	Expected	11.7 \pm 4.8	0.29	Additive	8.3 \pm 4.0	3.17	Additive
	Observed	13.3 \pm 4.2			13.3 \pm 7.1		
Clothianidin	Expected	10.0 \pm 3.7	0.0	Additive	16.7 \pm 3.3	3.37	Additive
	Observed	8.3 \pm 1.7			6.7 \pm 3.3		
<i>Heterorhabditis</i> sp. NYSAES Nema 2							
Imidacloprid	Expected	11.7 \pm 4.8	0.12	Additive	8.3 \pm 4.0	0.0	Additive
	Observed	13.3 \pm 4.2			13.3 \pm 7.1		
Clothianidin	Expected	10.0 \pm 3.7	3.00	Additive	16.7 \pm 3.3	3.67	Additive
	Observed	8.3 \pm 1.7			6.7 \pm 3.3		
<i>P. popilliae</i>							
Imidacloprid	Expected	16.7 \pm 5.6	10.02	Antagonistic	13.3 \pm 3.3	9.77	Antagonistic
	Observed	3.3 \pm 2.1			1.7 \pm 1.7		
Clothianidin	Expected	8.3 \pm 4.0	0.64	Additive	16.7 \pm 5.6	0.41	Additive
	Observed	10.0 \pm 3.7			8.3 \pm 3.1		
<i>Bt</i> var. <i>galleriae</i>							
Imidacloprid	Expected	10.0 \pm 4.4	6.94	Antagonistic	6.7 \pm 3.3	1.67	Additive
	Observed	1.7 \pm 2.1			3.3 \pm 1.7		
Clothianidin	Expected	6.7 \pm 4.2	0.41	Additive	16.7 \pm 7.6	3.79	Additive
	Observed	8.3 \pm 4.0			8.3 \pm 5.4		
<i>Bt</i> var. <i>tenebrionis</i>							
Imidacloprid	Expected	11.7 \pm 4.0	5.79	Antagonistic	8.3 \pm 3.0	2.91	Additive
	Observed	3.3 \pm 2.1			3.3 \pm 3.3		
Clothianidin	Expected	8.3 \pm 4.0	3.17	Additive	16.7 \pm 5.8	3.22	Additive
	Observed	13.3 \pm 4.9			5.0 \pm 2.2		

^a Observed = efficacy of both control agents applied at the same time. Expected = sum of efficacy of each control agent applied separately.

^b 1/2 = (0.225 kg AI/ha) half of the high label rate recommendation for white grub control.

^c 1/4 = (0.123 kg AI/ha) quarter of the high label rate recommendation for white grub control.

arapu et al. (2007), for instance, reported a synergism in *A. orientalis* for *H. bacteriophora* combined with a low dose (84 mg AI/ha) of imidacloprid but not with a high dose (168 mg AI/ha). Koppenhöfer et al. (2000a) reported an additive effect in *P. japonica* and *A. orientalis* when imidacloprid was combined with a low dose (1.25×10^9 IJ/ha) of *H. bacteriophora*, but an antagonistic effect with a high dose (2.5×10^9 IJ/ha). Nematode strain might also be related to diverging results because our evaluations featured a commercial strain of *H. bacteriophora* (Heteromask), while Koppenhöfer et al. (2000b) used non-commercial strains (TF and NC1).

4.2. Mechanisms and neonicotinoids effects

Our results did not allow us to ascertain which insecticide was synergized and which was the synergist. But due to their disruptive behavioral effects, neonicotinoids have been studied for their

capacity to synergize the effects of other stressors, in particular natural enemies. First, neonicotinoids act as an antifeedant. This attribute may not impact white grubs in late fall as they prepare to overwinter, but it could be relevant in early spring when larvae reemerge to feed before pupation (Grewal et al., 2001). As another dimension, if ingestion is decreased, then the insect may not acquire lethal doses of systemic insecticides (e.g. neonicotinoids) or enterobacteria (e.g. *Bt* and *P. popilliae*). Second, neonicotinoids interfere with the insect nervous system to produce uncoordinated movements, tremor and paralysis. This can disrupt their ability to descend in the soil profile to avoid freezing temperatures in winter or to avoid exposure to pathogens, parasitoids and predators (Ehler, 1998; Grewal et al., 2001). Third, disruption of normal nerve function as a direct effect of the neonicotinoid on the cholinergic receptors in the postsynaptic membrane could interfere with normal defensive behaviors (Koppenhöfer et al., 2000b). In response to

Table 4
Laboratory mortality (mean \pm SE) of third instar *Amphimallon majale* and the interaction among different combinations of biological and neonicotinoid insecticides at 30 DAT in 2006.

Treatment	Measurement ^a	Rate 1/2 ^b			Rate 1/4 ^c		
		Mortality	X ²	Effect	Mortality	X ²	Effect
<i>B. bassiana</i> GHA	Imidacloprid	Observed	20.0 \pm 2.6	2.88	Additive	28.3 \pm 4.7	0.14
		Expected	31.7 \pm 7.0				
	Clothianidin	Observed	21.7 \pm 5.4	7.18	Antagonistic	21.7 \pm 5.4	4.08
		Expected	40.0 \pm 6.3				
<i>M. anisopliae</i> Met F52	Imidacloprid	Observed	6.7 \pm 3.3	4.63	Antagonistic	11.7 \pm 1.6	0.0
		Expected	15.0 \pm 3.4				
	Clothianidin	Observed	5.0 \pm 2.2	14.40	Antagonistic	1.7 \pm 1.7	16.81
		Expected	23.3 \pm 3.3				
<i>M. anisopliae</i> NYSAES	Imidacloprid	Observed	31.7 \pm 7.0	1.40	Additive	48.3 \pm 7.9	3.69
		Expected	43.3 \pm 4.2				
	Clothianidin	Observed	41.7 \pm 10.7	0.25	Additive	25.0 \pm 6.2	7.32
		Expected	51.7 \pm 4.0				
<i>H. bacteriophora</i>	Imidacloprid	Observed	58.3 \pm 3.1	13.45	Synergistic	48.3 \pm 3.1	6.30
		Expected	40.0 \pm 4.5				
	Clothianidin	Observed	58.3 \pm 3.0	5.90	Synergistic	51.7 \pm 4.0	3.40
		Expected	48.3 \pm 4.0				
<i>P. popilliae</i>	Imidacloprid	Observed	16.7 \pm 3.3	3.55	Additive	30.0 \pm 8.5	1.83
		Expected	28.3 \pm 3.1				
	Clothianidin	Observed	18.3 \pm 3.1	6.90	Antagonistic	23.3 \pm 2.1	1.75
		Expected	36.7 \pm 4.2				
<i>Bt</i> var. <i>galleriae</i>	Imidacloprid	Observed	25.0 \pm 4.3	0.10	Additive	26.7 \pm 6.1	1.86
		Expected	25.0 \pm 4.3				
	Clothianidin	Observed	13.3 \pm 3.3	10.07	Antagonistic	28.3 \pm 6.5	0.01
		Expected	33.3 \pm 3.3				
<i>Bt</i> var. <i>tenebrionis</i>	Imidacloprid	Observed	20.0 \pm 5.2	2.16	Additive	26.7 \pm 5.6	7.22
		Expected	30.0 \pm 5.2				
	Clothianidin	Observed	23.3 \pm 4.9	3.79	Additive	35.0 \pm 5.6	1.33
		Expected	38.3 \pm 6.5				

^a Observed = efficacy of both control agents applied at the same time. Expected = sum of efficacy of each control agent applied separately.

^b 1/2 = (0.225 kg AI/ha) half of the high label rate recommendation for white grub control.

^c 1/4 = (0.123 kg AI/ha) quarter of the high label rate recommendation for white grub control.

the presence of *H. bacteriophora*, the frequency of grooming behaviors like brushing, chewing and rubbing have been shown to increase (Koppenhöfer et al., 2000a). A reduction in those activities would likely disrupt this defense against nematodes, but would unlikely impair defense from fungi or bacteria where a cellular and humeral immune response is the main defense (Narayanan, 2004).

4.3. Antagonistic interactions involving *Bt* and fungi

Beyond the synergistic interactions detected in this study, consistent antagonistic interactions were also detected among bacterial combinations in the laboratory. Of the 24 total combinations tested across the three bacterial products, 8 and 11 were antagonistic against *A. majale* and *P. japonica*, respectively. Those combinations included all three bacteria products, both white grub species and the higher dose (1/2) of both neonicotinoids. In a field study on *P. popilliae*, 14 chemical pesticides (herbicides, fungicides and insecticides) were shown to reduce levels of spore viability, spore germination and/or vegetative cell growth (Dingman, 1994). Therefore chemical insecticides could have inhibitory effects on bacteria and their prevalence in the field. The mechanism, however, is unclear. When combinations of *Bt*-toxin and thiodicarb (a carbamate insecticide) were studied, the interaction was syner-

gistic at a high thiodicarb and low *Bt*-toxin concentration against *Helicoverpa armigera* Huebner, but all other combinations were antagonistic (Khaliq and Amhed, 2005). We are aware of no scientific literature that addresses the interactions between neonicotinoids and *Bt* toxins, spores or products.

The only other antagonistic interactions detected were with some combinations of entomopathogenic fungi and both neonicotinoids against *A. majale*. Entomopathogenic fungi may be incompatible with chemical insecticides due to lower germination rate, decreased production of enzymes necessary for penetration of the insect's cuticle, and poor mycelium growth ratio (Bednarek et al., 2004). For instance, mycelium growth ratios of *B. brongniartii* and *B. bassiana* are inhibited by carbosulfan (Bednarek et al., 2004). The same study showed that growth of *B. brongniartii* was stimulated by carbofuran. As another example, when thiamethoxam and imidacloprid were examined with respect to their compatibility with *B. bassiana* and *M. anisopliae*, thiamethoxam was compatible with both fungi, while imidacloprid was moderately toxic to both fungi (Battista Filho et al., 2001). At least in the case of entomopathogenic fungi, the type of interaction is therefore idiosyncratic; whether a combination is antagonistic depends on the specific fungus species and the specific insecticide. Antagonistic interactions in white grubs have been reported before, but all feature nematodes. For instance, thiamethoxam combined with *H. bacteriophora* was antagonistic

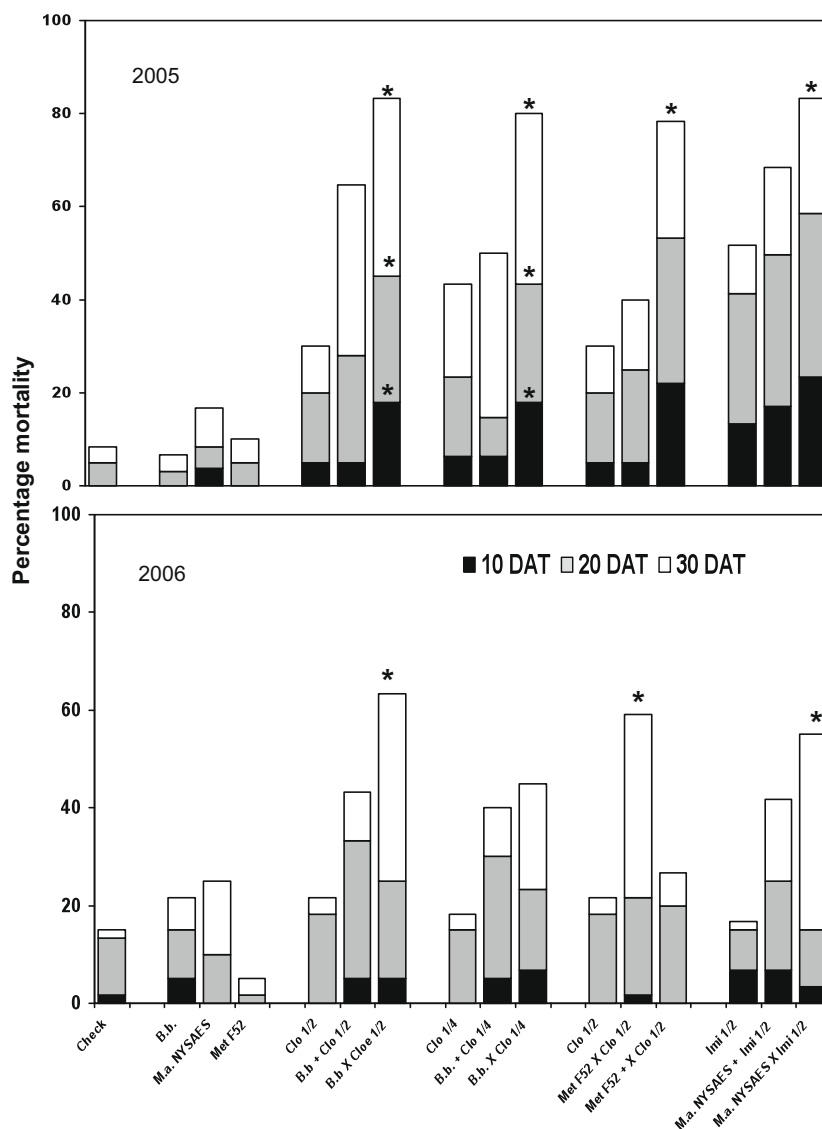


Fig. 1. Percent mortality of *Popillia japonica* at 10, 20 and 30 days after treatment (DAT) with different insecticide combinations under laboratory conditions. B.b. = *Beauveria bassiana*, M.a. = *Metarhizium anisopliae*, Clo-1/4 = clothianidin quarter label rate (0.123 kg AI/ha), Clo-1/2 = clothianidin half label rate (0.225 kg AI/ha), Imi-1/2 = imidacloprid half label rate (0.225 kg AI/ha), + = expected mortality based on mortalities of both insecticides applied separately, x = mortality observed when both insecticides are applied at the same time, * = significant synergistic interaction between biological and chemical insecticide (χ^2 test value for 1 df).

against *A. majale*, *A. orientalis*, *M. castanea* and *P. japonica* under field conditions (Koppenhöfer et al., 2002).

4.4. Strength of synergies diminishes from laboratory to field

For *P. japonica*, laboratory mortality in treatments with synergistic interactions was 2.2 times higher than the greenhouse and 6.0 times higher than the field. The equivalent values for *A. majale* were 1.6 and 1.8 times higher. The decline in mortality from laboratory to greenhouse to field might be linked to the size of the experimental units. Laboratory bioassay cups were 33 times smaller than greenhouse pots and 185 times smaller than field arenas. Even if application rates were the same proportional to area, larvae would have had more opportunities to avoid contaminated soil in the larger arenas. Previous studies have reported evasive behavior in *P. japonica* to soil treated with *M. anisopliae* (Villani et al., 1994; Fry et al., 1997). Evasive behavior has also been observed in *P. japonica* with imidacloprid and in *A. majale* with both imidacloprid and *M. anisopliae* (Morales, unpublished data).

The strength of synergistic interactions from lab to field might also diminish due to variation in environmental conditions and its impact on white grub behavior. From the laboratory to the greenhouse, temperature and humidity fluctuations increased considerably. While even more variation was expected in the field, it may be that low temperatures in late fall had an inhibitory effect on the insecticides. The average temperature at 7 DAT was 6.9 °C (3.3–10.5 °C) for *P. japonica* and 9.6 °C (5.5–13.8 °C) for *A. majale*. At least for biological insecticides, low temperatures can inactivate or otherwise affect performance. The optimal range is 20–30 °C for *B. bassiana* and *M. anisopliae* and 15–30 °C for *H. bacteriophora* (Bruck et al., 2008; Pandey, 2008).

Low air temperature in fall may also have affected the recovery of *P. japonica* larvae if they responded by moving down in the soil profile. This may explain why so few larvae were collected at the 30 DAT evaluation in late fall, compared to 174 DAT with higher temperatures and twice as many larvae recovered from the untreated checks. *Amphimallon majale* tolerates colder surface soil conditions than *P. japonica* before moving down in the soil profile

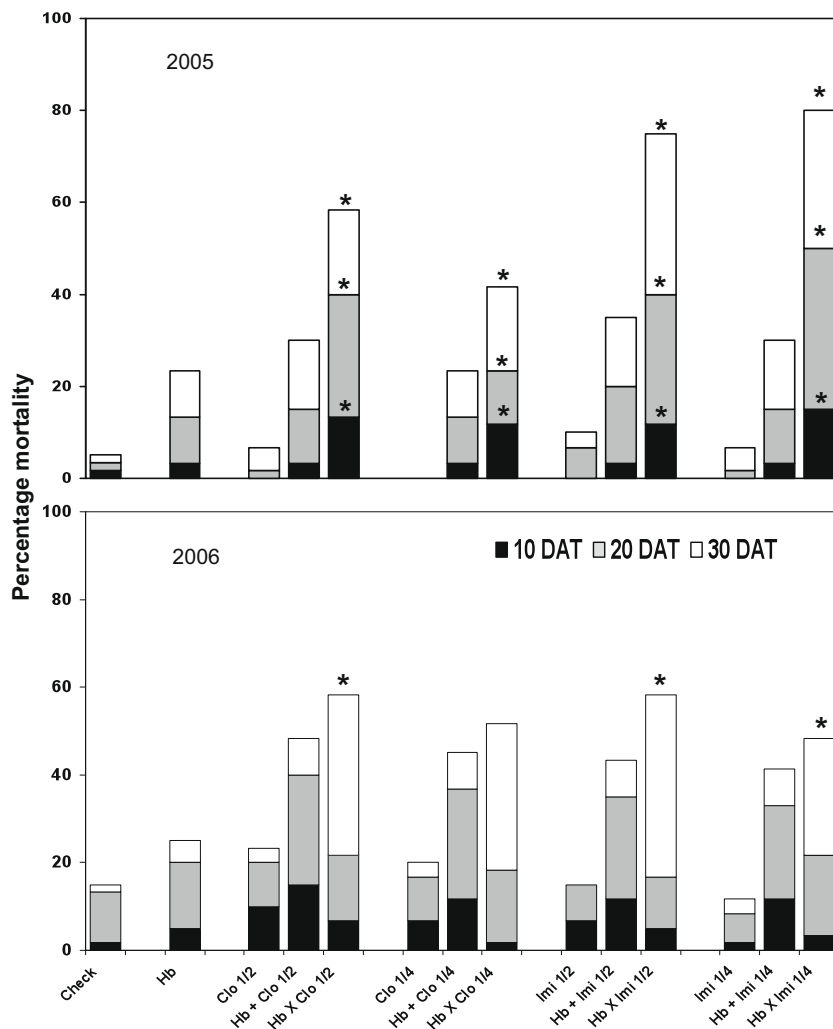


Fig. 2. Percent mortality of *Amphimallon majale* at 10, 20 and 30 days after treatment (DAT) with different insecticide combinations under laboratory conditions. H.b. = *Heterorhabditis bacteriophora*, Clo-1/4 = clothianidin quarter label rate (0.123 kg AI/ha), Clo-1/2 = clothianidin half label rate (0.225 kg AI/ha), Imi-1/4 = imidacloprid quarter label rate (0.123 kg AI/ha), Imi-1/2 = imidacloprid half label rate (0.225 kg AI/ha), + = expected mortality based on mortalities of both insecticides applied separately, x = mortality observed when both insecticides are applied at the same time, * = significant synergistic interaction between biological and chemical insecticide (X^2 test value for 1 df).

(Morales, personal observation). For this reason an effect of overwintering on the strength of treatment effects could only be tested for *A. majale*.

4.5. Implications for soil insect pest management

Validation and implementation of biological-neonicotinoid synergies as a new approach for turfgrass IPM programs still face major hurdles. Foremost is the relatively low level of control currently attainable; adoption will be extremely limited if field efficacy cannot surpass 70%. Another hurdle is the limited availability of biological products. Only two entomopathogenic fungi products, for instance, are registered in the United States for control of white grubs in turfgrass. Among entomopathogenic nematode products, limitations are posed by specificity of host-pathogen interactions, formulation challenges, and variability in product and field results. Due to differential effects among white grub species reported here for combined products, and reported previously for individual products (Morales-Rodriguez et al., accepted for publication), a further challenge is that pest management practitioners will need to diagnose and differentiate scarab species before any intervention.

Finally, costs of interventions might pose an additional hurdle, especially if scouting is contemplated and if two separate applications are required. As an alternative for curative control, however, synergistic interaction products would reduce costs in the sense that applications rates of the chemical synergist would be reduced. Moreover, the late season applications targeting large third instars would open opportunities to make applications over a smaller area based on scouting and threshold assessment. Multiyear research has shown that interventions against *A. majale* may be warranted only 20% of the time in both home lawn and golf course settings in New York (Nyrop et al., 1995). Tank mixes could be a feasible way to minimize application costs associated with such an approach, as certain combinations of chemical and biological insecticides can be mixed without affecting the quality of the biological agent or the synergistic effect (Neves et al., 2001; Koppenhöfer et al., 2003). The approach might also supplant reliance on chemical insecticides since it would broaden opportunities for biological alternatives beyond preventive control windows. Indeed, Koppenhöfer and Fuzy (2008) propose early curative control because nematode and neonicotinoid combinations could be more effective against second and early third instars than late third instars.

Table 5

Greenhouse mortality (mean \pm SE) of third instar *Popillia japonica* and *Amphimallon majale* and the interaction among different combinations of biological and neonicotinoid insecticides at 30 DAT.

Treatment ^a	Measurement ^b	2006			2007		
		Mortality	χ^2	Effect	Mortality	χ^2	Effect
<i>P. japonica</i>							
<i>B. bassiana</i>							
Clothianidin 1/2	Observed	29.5 \pm 5.6	9.36	Synergistic	53.0 \pm 4.1	13.78	Synergistic
	Expected	17.5 \pm 3.0			44.0 \pm 4.5		
Clothianidin 1/4	Observed	21.5 \pm 3.1	0.00	Additive	40.0 \pm 4.9	0.96	Additive
	Expected	15.0 \pm 3.3			37.7 \pm 6.7		
<i>M. anisopliae</i> Met F52							
Clothianidin 1/2	Observed	28.5 \pm 3.7	19.50	Synergistic	13.0 \pm 4.4	0.03	Additive
	Expected	17.0 \pm 3.6			30.0 \pm 3.6		
<i>M. anisopliae</i> NYSAES							
Imidacloprid 1/2	Observed	18.0 \pm 4.7	0.98	Additive	34.0 \pm 4.0	0.44	Additive
	Expected	14.5 \pm 4.4			33.0 \pm 4.7		
<i>A. majale</i>							
<i>H. bacteriophora</i>							
Clothianidin 1/2	Observed	32.0 \pm 3.3	12.54	Synergistic	36.0 \pm 5.8	14.80	Synergistic
	Expected	18.0 \pm 3.6			20.0 \pm 5.2		
Clothianidin 1/4	Observed	22.0 \pm 3.6	1.19	Additive	18.0 \pm 3.8	0.39	Additive
	Expected	18.0 \pm 2.0			22.0 \pm 4.4		
Imidacloprid 1/2	Observed	40.0 \pm 7.5	37.50	Synergistic	42.0 \pm 7.6	34.58	Synergistic
	Expected	16.0 \pm 2.7			18.0 \pm 5.5		
Imidacloprid 1/4	Observed	51.0 \pm 6.0	97.79	Synergistic	58.0 \pm 4.7	94.32	Synergistic
	Expected	14.0 \pm 3.1			19.0 \pm 4.7		

^a 1/2 = (0.225 kg AI/ha) half of the high label rate recommendation for white grub control; 1/4 = (0.123 kg AI/ha) quarter of the high label rate recommendation for white grub control.

^b Observed = efficacy of both control agents applied at the same time. Expected = sum of efficacy of each control agent applied separately.

Table 6

Field mortality (mean \pm SE) of third instar *Popillia japonica* and *Amphimallon majale* and the interaction among different combinations of biological and neonicotinoid insecticides at 30 DAT.

Treatment ^a	Measurement ^b	30 DAT			174 DAT		
		Mortality	χ^2	Effect	Mortality	χ^2	Effect
<i>P. japonica</i>							
<i>B. bassiana</i>							
Clothianidin 1/2	Observed	0.0 \pm 0.0	0.00	Additive	20.6 \pm 4.9	8.25	Antagonistic
	Expected	0.0 \pm 0.0			42.6 \pm 5.5		
Clothianidin 1/4	Observed	0.0 \pm 0.0	0.30	Additive	23.5 \pm 4.0	3.28	Additive
	Expected	0.3 \pm 0.2			36.8 \pm 5.0		
<i>M. anisopliae</i> Met F52							
Clothianidin 1/2	Observed	0.0 \pm 0.0	0.00	Additive	20.6 \pm 6.8	2.83	Additive
	Expected	0.0 \pm 0.0			32.4 \pm 5.2		
<i>M. anisopliae</i> NYSAES							
Imidacloprid 1/2	Observed	0.0 \pm 0.0	0.00	Additive	26.5 \pm 2.9	0.14	Additive
	Expected	0.0 \pm 0.0			26.5 \pm 4.3		
<i>A. majale</i>							
<i>H. bacteriophora</i>							
Clothianidin 1/2	Observed	15.9 \pm 9.5	0.29	Additive	42.3 \pm 11.0	7.98	Synergistic
	Expected	14.4 \pm 5.6			30.0 \pm 3.6		
Clothianidin 1/4	Observed	7.6 \pm 4.7	4.20	Additive	36.5 \pm 10.8	0.76	Additive
	Expected	16.5 \pm 4.0			34.6 \pm 5.6		
Imidacloprid 1/2	Observed	18.2 \pm 6.7	1.47	Additive	45.4 \pm 16.7	16.10	Synergistic
	Expected	26.9 \pm 4.3			26.9 \pm 4.3		
Imidacloprid 1/4	Observed	22.0 \pm 6.8	0.00	Additive	48.1 \pm 11.6	20.70	Synergistic
	Expected	23.2 \pm 7.2			26.9 \pm 8.6		

^a 1/2 = (0.225 kg AI/ha) half of the high label rate recommendation for white grub control; 1/4 = (0.123 kg AI/ha) quarter of the high label rate recommendation for white grub control.

^b Observed = efficacy of both control agents applied at the same time. Expected = sum of efficacy of each control agent applied separately.

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