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Beauveria bassiana and Elisa Determination of Neonicotinoids to Improve Management of *Listronotus maculicollis*

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BEAUVERIA BASSIANA AND ELISA DETERMINATION OF NEONICOTINOIDS
TO IMPROVE MANAGEMENT OF *LISTRONOTUS MACULICOLLIS*

BY

CHRISTOPHER D. CLAVET

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE

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OF

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ABSTRACT

The “annual bluegrass weevil” (*Listronotus maculicollis*) became resistant to synthetic pyrethroid insecticides (bifenthrin and cyhalothrin) in several adult weevil populations from Connecticut, Rhode Island, and Massachusetts in 2007-09, and management of this insect has become increasingly complex. Annual bluegrass weevil continues to be a serious pest of *Poa annua* L. (Poaceae) and bentgrasses (*Agrostis* spp) on many golf courses in mid-Atlantic and northeastern United States and eastern Canada. Adults chew notches on grass blades and at the juncture of leaves and stems. However, adult feeding has little effect on plant vitality. Early instars feed inside plant stems and late instars on plant crowns. The first generation larvae, which usually become apparent in late May or early June, typically cause the most severe damage. During July and August damage caused by second generation larvae is usually less extensive, especially if good control of the overwintering generation was obtained. In most cases, adequate control of the insect has been achieved through the use of pyrethroid applications targeting adult weevils as they emerge from overwintering sites and before they begin to lay eggs. However, if a population is resistant to pyrethroids, alternative controls are required to prevent damage. My research objectives were to evaluate the entomopathogenic fungus *Beauveria bassiana* for control of *L. maculicollis* and how neonicotinoid insecticides can best be used to manage this increasingly serious pest.

While pyrethroids remain the preferred choice of many golf course superintendents for managing this species, resistance has forced some superintendents to incorporate other strategies. Some of the new strategies include: (1) the use of a pyrethroid or chlorpyrifos early against overwintering adults; (2)

neonicotinoid/pyrethroid combinations (Aloft, Allectus) during peak adult emergence to control adults and first generation larvae; (3) primarily preventative larvicidal compounds (chlorantraniliprole (Acelepryn), neonicotinoids) for early instars; and (4) curative larvicidal compounds (trichlorfon (Dylox), spinosad (Conserve), indoxacarb (Provaunt), chlorpyrifos, pyrethroids) for control of fourth and fifth instars. Some locations may need to use one or more of these strategies to prevent turf damage and resistance development. It is imperative that the timing of treatments coincide with various life stages (adults, early or late instar larvae) to maximize chemical efficacy. This is particularly important for the systemic compounds (neonicotinoids / chlorantraniliprole) to insure there is sufficient chemical in the xylem for maximum effectiveness. If treatment strategies 1-3 are not effective, a curative larvicidal compound may need to be applied to prevent damage. Finally, since all subsequent generations come from the overwintering adults, it is imperative that a superintendent control those adults and any larvae that they produce (1st generation).

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I thank my advisor, Dr. Steven Alm for his advice and support over the years and throughout this process. Without his guidance and mentoring none of this would have been possible. I also thank my committee members Drs. Richard Casagrande and Jose Amador and defense chair, Dr. Pat Logan. Throughout this process, the support and care provided by the Requintina family has been invaluable and I thank them. Also, a special thanks to: Emily Hampton, Olivia Barsoian, Carissa Koski, Nicholas Caldarelli, and Patrick McNiece. I thank my family and friends for their support and encouragement throughout this process. To Meghan, thank you for your support, patience, and care.

PREFACE

The chapters of this thesis are being submitted in manuscript format. Chapter one, “Laboratory Assessment of *Beauveria bassiana* strain GHA for Control of *Listronotus maculicollis* (Coleoptera: Curculionidae) Adults” has been accepted by the J. of Economic Entomology with co-authors Emily Hampton, Matthew Requintina and Steven R. Alm. Chapter two, “Determining the Amounts of Clothianidin and Imidacloprid in *Poa annua* (L.) by ELISA and Their Effects With and Without Bifenthrin on *Listronotus maculicollis* (Coleoptera: Curculionidae)” will also be submitted to the J. of Economic Entomology with co-authors Emily Hampton, Matthew Requintina, Richard S. Cowles, Frank J. Byrne and Steven R. Alm.

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CHAPTER 1

**“Laboratory Assessment of *Beauveria bassiana* strain GHA for Control of
Listronotus maculicollis (Coleoptera: Curculionidae)”**

by

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Accepted by J. Economic Entomology

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ABSTRACT

Bioassays were designed to evaluate *Beauveria bassiana* (Balsamo) Vuillemin strain GHA against *Listronotus maculicollis* (Kirby) adults. *B. bassiana* and its “inert” carrier oil in the product BotaniGard and the “inert” carrier oil alone provided 99 and 96% mortality respectively in Petri dish assays 1 d after treatment when applied in 1 ml water. When the same treatments were applied in 0.5 ml of carrier water mortality was only 1.4 and 0.7% respectively 1 d after treatment. After 10 d in Petri dishes, *B. bassiana* and its “inert” carrier oil and the “inert” carrier oil alone applied in 0.5 ml water showed 77 and 9% mortality respectively. When one-tenth the label dosage of *B. bassiana* and “inert” carrier oil was combined with neonicotinoids clothianidin, imidacloprid, and dinotefuran applied in 1 ml water, there were significant increases (34, 30, and 68% respectively) in weevil mortality over the neonicotinoids alone 1 d after treatment. When one-tenth the label dosage of “inert” carrier oil alone was combined with neonicotinoids clothianidin, imidacloprid, and dinotefuran applied in 1 ml water, there were also significant increases with clothianidin and dinotefuran (38 and 24% respectively) in weevil mortality over the neonicotinoids alone 1 d after treatment. *B. bassiana* and its “inert” carrier oil provided 28, 50, and 78% mortality at the highest label dosage and 47, 76, and 89% mortality at 4× the highest label dosage in turf plug assays at 7, 10, and 14 d after treatment. Addition of 5 or 20% MycoMax (a nutrient source for *B. bassiana*) did not significantly increase mortality in turfgrass plug assays.

INTRODUCTION

Larvae of *Listronotus maculicollis* (Kirby) (Coleoptera: Curculionidae) are the most destructive insect pests of *Poa annua* L. (Poales: Poaceae) on golf courses in the northeastern United States (Vittum et al. 1999). This insect species was first seen damaging turfgrass in Connecticut in 1931 and by the late 1950s and early 1960s was responsible for severe damage on golf courses in the state (Britton 1932, Tashiro 1976). Adult *L. maculicollis* chew notches on grass blades at the juncture of leaves and stems. Adult damage is not as severe as larval feeding. Larval feeding can result in extensive turf damage and death since they feed at the plant crown. Where larval densities exceed 450 per 929 cm² (1 ft²), injury to golf course greens, collars, and fairways is common (Watschke et al. 1994). Instars 1-3 feed inside plant stems while 4th and 5th instars feed on plant crowns. There are normally two to three generations of *L. maculicollis* per year in the northeastern U. S.

Fourth generation pyrethroids provided excellent control of weevils in the 1990s and early 2000s. These products were principally used to target adult weevils as they colonized turf after overwintering in areas surrounding tees, greens, and fairways. In 2005, the first indications of diminished pyrethroid effectiveness were reported (Vittum 2005). In 2009 the first study to confirm pyrethroid resistance was published (Ramoutar 2009a), and two subsequent studies (Ramoutar et al. 2009b; 2010) further confirmed pyrethroid resistance.

Alternative controls are needed to manage this serious pest. *Beauveria bassiana* (Balsamo) Vuillemin is an important biological control agent for many insect pests

(Fargues and Luz 2000; Furlong and Groden 2001; Quintela and McCoy 1998). In this study we evaluated a commercially available formulation of *B. bassiana* against *L. maculicollis* adults in Petri dish assays and simulated field studies with turfgrass plugs both with and without neonicotinoid insecticides and a nutrient source for *B. bassiana* called MycoMax. We concentrated on adult control because instars 1-3 are protected inside plant stems and can only be controlled with systemic insecticides and by the time 4th and 5th instars emerge, the majority of damage has occurred. Furthermore, adult population densities are more readily monitored than larval stages.

METHODS

Petri Dish Assays. Adult weevils were collected from different golf courses and placed on 9 cm diameter filter paper discs in 100 × 15 mm Petri dishes treated with various dosages of *Beauveria bassiana* strain GHA and its “inert” carrier oil (BotaniGard, Laverlam International, Butte, MT), the “inert” carrier oil alone, and neonicotinoid insecticides and combinations with BotaniGard and its “inert” carrier oil applied in 0.5 or 1 ml water. Controls consisted of treating filter paper discs with 1 ml of water and adding adult weevils. Petri dishes were wrapped in parafilm to maintain humidity and prevent weevils from escaping. Assays were rated for adult mortality for up to 10 d. In the first set of assays, we evaluated the highest label dosage of *B. bassiana* strain GHA (25.46 liters/ha) and the “inert” carrier oil filtered 2× (Millex HV 0.45 µm then MillexVV 0.1µm, EMD Millipore Corp., Billerica, MA) at 25.46 liters/ha 1, 3, 5, 7, and 10 d after treatment. Six separate experiments with weevils from three

locations (Westerly, RI; Baltic and Norwich, CT) were combined for analysis (10 weevils per replicate, 14 replicates, 140 weevils for each treatment).

In a second assay, we evaluated three neonicotinoid insecticides: imidacloprid (Bayer Environmental Science, Research Triangle Park, NC), clothianidin (Valent U. S. A. Corp, Walnut Creek, CA) and dinotefuran (PBI – Gordon, Kansas City, MO) at label dosages with and without one-tenth the label dosage of *B. bassiana* strain GHA and its “inert” carrier oil and 88.7% of one-tenth the label dosage of the “inert” carrier oil alone for adult mortality 1, 3, 5, and 7 d after treatment. One experiment was conducted with five replicates with weevils from Westerly, RI (10 weevils per replicate, 5 replicates, 50 weevils per treatment).

Turf Plug Assays. In another series of experiments, we evaluated the highest label dosage of BotaniGard (25.46 liters/ha); however, mortality was not high enough to be able to recommend it as a control option. Therefore, we compared the highest label dosage and 4× the highest label dosage (101.84 liters/ha) to explore a dosage response, and to determine whether mortality would be comparable to standard synthetic insecticides. Controls consisted of treating 5.72 cm diameter turfgrass plugs with water only and adding ten weevils. Turfgrass plugs were treated using a CO₂ powered sprayer equipped with an 8002EVS TeeJet nozzle (Spraying Systems Co., Wheaton, IL). After treatment, turfgrass plugs were placed in 147 ml plastic cups where the weevils were added and then contained with a 1 mm mesh screen. Three assays using weevils from two locations (Westerly, RI and Norwich, CT) (10 weevils per plug, 4 replicates, 120 weevils per treatment) were rated for adult mortality 7, 10 and 14 d after treatment and combined for analysis.

In another set of turf plug assays, adult weevils (5 per plug, 4 replicates, 20 weevils per treatment) were collected from Westerly and Pawtucket, RI and placed on turfgrass plugs treated with the highest label dosage (25.46 liters/ha) of *B. bassiana* strain GHA in its “inert” carrier oil both with and without 5 and 20% MycoMax (a sweet whey designed to be a nutrient source for *B. bassiana* obtained from Dr. Scott Costa, Univ. of VT) in volumes of water equal to 815 liters/ha (2 gal/1,000 ft²). Controls consisted of treating turfgrass plugs with water only and adding five weevils.

Statistical Analysis. Percent mortalities were transformed by taking the arcsine of the square root of the proportion before ANOVA and mean separation via Tukey’s HSD test (SAS version 9.2). Untransformed means and errors are shown in figures.

RESULTS

Petri Dish Assays. There was significant mortality of adults in Petri dish assays at the highest BotaniGard label dosage and the “inert” carrier oil filtered 2× at the same dosage at 1 d ($F = 691.70$; $df = 4,52$; $P < 0.01$), 3 d ($F = 494.96$; $df = 4,52$; $P < 0.01$), 5 d ($F = 392.62$; $df = 4,52$; $P < 0.01$), 7 d ($F = 395.79$; $df = 4,52$; $P < 0.01$), and 10 d ($F = 252.61$; $df = 4,52$; $P < 0.01$) after treatment (Fig. 1). We also demonstrated with Petri dishes assays that using 0.5 versus 1.0 ml of application water with 16.19 µl BotaniGard or 16.19 µl “inert” carrier oil alone per 9 cm diameter filter paper disc (= 25.46 liters/ha) was the difference between low and excellent control, respectively (Fig. 1).

There was significant mortality of adults in Petri dish assays with BotaniGard at the label dosage (25.46 liters/ha) and label dosages of clothianidin and dinotefuran with one-tenth the label dosage of BotaniGard (2.54 liters/ha) and 88.7% of one-tenth the

label dosage of “inert” carrier oil alone in Petri dish assays ($F = 30.34$; $df = 6,24$; $P < 0.01$; and $F = 86.80$; $df = 6,24$; $P < 0.01$ respectively) 1 d after treatment (Figs. 2, and 4). There was significant mortality of adults in Petri dish assays with label dosages of imidacloprid with one-tenth the label dosage of BotaniGard (2.54 liters/ha) 1 d after treatment in Petri dish assays ($F = 40.18$; $df = 6,24$; $P < 0.01$) (Fig. 3). Clothianidin, imidacloprid and dinotefuran alone at the label dosage also caused significant mortality of adults 3 d after treatment (Figs. 2, 3 and 4).

Turf Plug Assays. When adults were placed on treated turfgrass plugs there was significant mortality at the highest and 4× the highest label dosage 7 ($F = 23.14$; $df = 2,22$; $P < 0.01$), 10 ($F = 40.77$; $df = 2,22$; $P < 0.01$) and 14 ($F = 17.44$; $df = 2,22$; $P < 0.01$) d after treatment (Fig. 5). Four times the highest label dosage caused faster and greater mortality (Fig. 5).

Addition of 5 or 20% MycoMax (an adjuvant providing sweet whey as a nutrient source for *B. bassiana*) did not significantly increase mortality 7 d ($F = 0.76$; $df = 2,14$; $P = 0.48$), 10 d ($F = 3.07$; $df = 2,14$; $P = 0.08$) or 14 d ($F = 2.29$; $df = 2,14$; $P = 0.14$) after treatment.

DISCUSSION

Petri dish assays at the highest label dosage (25.46 liters/ha) and the “inert” carrier oil filtered 2× at that dosage were very effective in causing mortality of adult weevils at 24 h. This indicates that mortality within 24 h was the result of the oil and not infection from *B. bassiana* strain GHA. When using oil formulations of entomopathogenic fungi there needs to be assurance that insecticidal activity is not the

result of the oil carrier (Goettel and Inglis 1997). Typical infection with entomopathogenic fungi such as *B. bassiana* will normally take several days to exert lethal effects which we did notice beginning on day five.

Cowles et al. (2000) used leaf dip bioassays with twospotted spider mites, *Tetranychus urticae* Koch and demonstrated the toxicity of trisiloxane surfactants, also considered inert ingredients. Toxicity was influenced by the leaf dip method which exaggerated the degree of wetting and indicated that high toxicity from surfactants was likely only in extremely wetted applications with high humidity. Although we were using “inert” carrier oil as a treatment and not a surfactant there was high mortality associated with a higher degree of wetness. This suggests that the Petri dish assay treatments of BotaniGard and its carrier oil using 1 ml of water may be effectively drowning the weevils similar to the activity of surfactants against *T. urticae* reported by Cowles et al. (2000).

The influence of moisture and humidity was also evident in the difference between treatments applied in volumes of 0.5 versus 1.0 ml of water. The “inert” carrier oil was ineffective when applied with 0.5 ml of water where the Petri dish was not as wet. However, when BotaniGard was applied in Petri dishes in 0.5 ml of water, significant mortality was evident starting at 5 d and increased at 7 and 10 d after treatment. This suggests that *B. bassiana* did begin to infect and kill adult *L. maculicollis* and that humidity and moisture were still high enough for infection even with 0.5 ml of water. This is supported by other experimental work that showed moisture was critical in effectiveness of *B. bassiana* strain GHA. Fargues and Luz (2000) found that the pathogenic activity of *B. bassiana* to *Rhodnius prolixus* Ståhl was

highly dependent on the moisture conditions and to a lesser extent on the temperature conditions. Their results showed a critical threshold of relative humidity between 95.5 and 97%.

One of the most encouraging results was the effect of one-tenth the label dosage of BotaniGard or the “inert” carrier oil with neonicotinoids (imidacloprid, clothianidin and dinotefuran). We noticed in other assays that neonicotinoids have a quick knockdown effect on adults; however, adults normally recover within 24 hours. Immobilizing adults for 24 hours may allow *B. bassiana* to overcome the insects’ defense mechanisms or the “inert” carrier oil is drowning adults. There was a significant difference in mortality between one-tenth the label dosage of BotaniGard and its “inert” carrier oil beginning on day five after treatment. Furlong and Groden (2001) found that significant synergism occurred in all instances where Colorado potato beetle, *Leptinotarsa decemlineata* (Say), larvae were exposed to imidacloprid before or simultaneously with *B. bassiana* treatment. They suggested that the synergism probably involves changes in the insect’s physiology that affects successful cuticular penetration or the initial proliferation of *B. bassiana* hyphal bodies within the host hemocoel. Quintela and McCoy (1998) found that the addition of imidacloprid to soil significantly impaired movement of larval *Diaprepes abbreviatus* (L.). When either *B. bassiana* or *Metarhizium anisopliae* (Metschnikoff) Sorokin were applied with imidacloprid, mortality and mycosis increased significantly. Surfactants in formulations of synthetic insecticides may also increase infection by *B. bassiana*.

Data on how quickly mortality of adults is achieved is important in management decisions since, if immediate control is needed to prevent damage, the highest label

dosage of BotaniGard may not act quickly enough to prevent damage. When adults were placed on treated turfgrass plugs, there was significant mortality at the highest and 4× the highest label dosage of BotaniGard 7, 10 and 14 d after treatment. Four times the highest label dosage did cause significantly faster and greater mortality at 7 and 10 d after treatment. Eventually at 14 d after treatment, the highest label dosage did cause significant mortality that was not significantly different from the 4× label dosage. The addition of MycoMax, a sweet whey adjuvant designed as a nutrient source for insect pathogenic fungi, did not significantly increase adult mortality.

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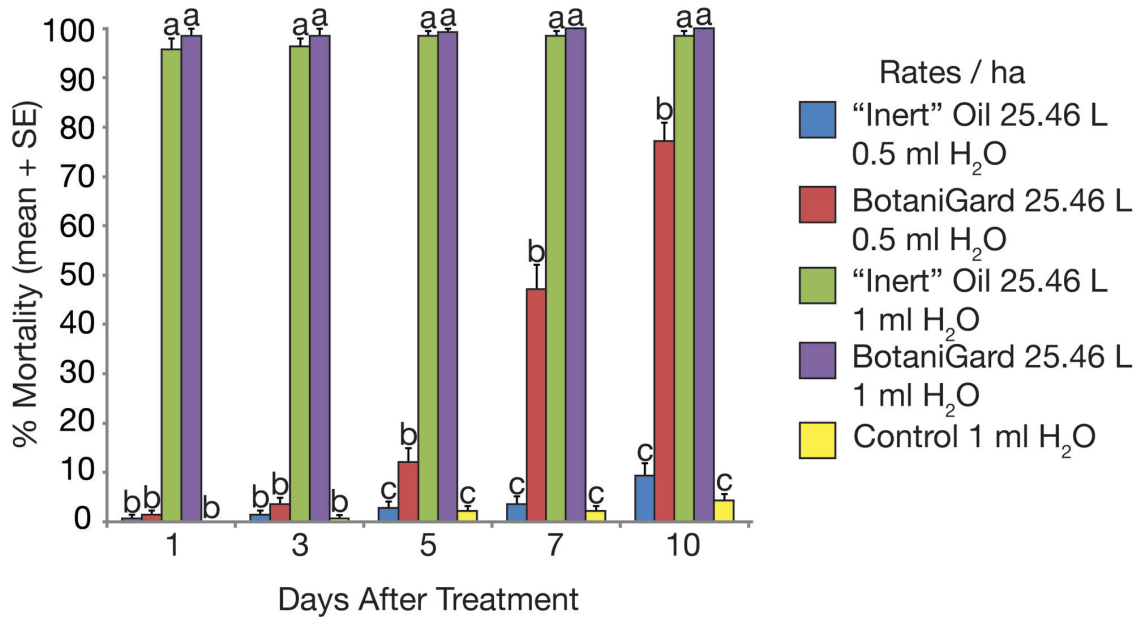


Figure 1: Percent mortality (mean + SE) of adults at the highest BotaniGard label dosage (25.46 liters/ha) and its carrier oil applied in 0.5 and 1.0 ml H₂O versus a water control in Petri dish assays, 1, 3, 5, 7 and 10 d after treatment. Means followed by the same letter are not significantly different ($P = 0.05$, Tukey's HSD test on arcsine transformed data).

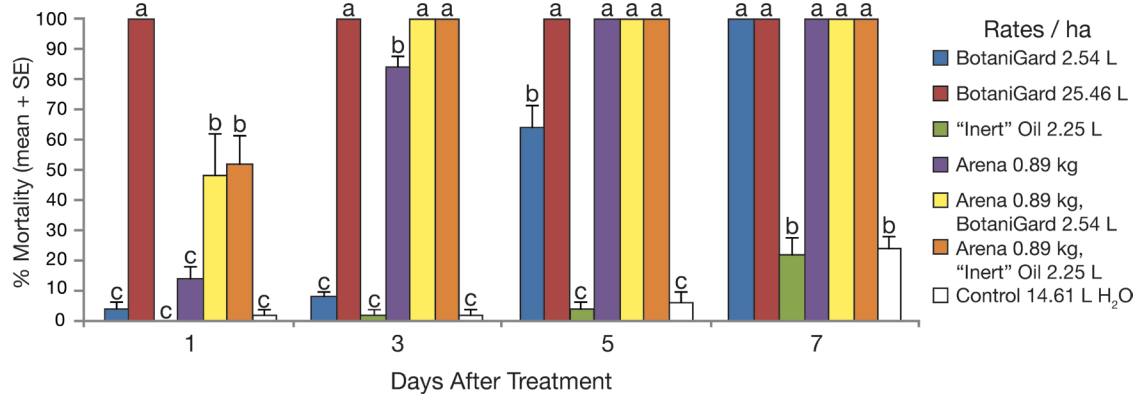


Figure 2: Percent mortality (mean + SE) of adult weevils to BotaniGard at one-tenth and the highest label dosage (2.54 and 25.4 liters/ha respectively), and label dosage of Arena (clothianidin) with and without one-tenth the label dosage of BotaniGard and its carrier oil in Petri dish assays 1,3, 5 and 7 d after treatment. Means followed by the same letter are not significantly different ($P = 0.05$, Tukey's HSD test on arcsine transformed data).

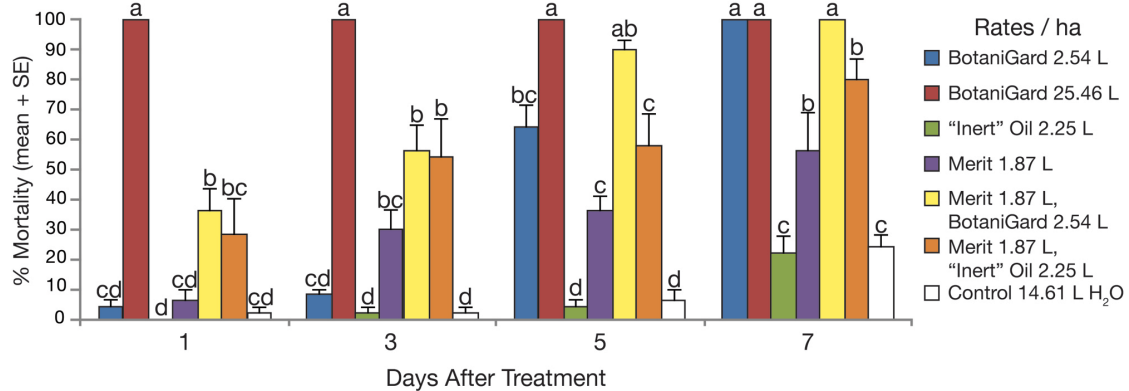


Figure 3: Percent mortality (mean + SE) of adult weevils to BotaniGard at one-tenth and the highest label dosage (2.54 and 25.4 liters/ha respectively), and label dosage of Merit (imidacloprid) with and without one-tenth the label dosage of BotaniGard and its carrier oil in Petri dish assays 1,3,5,and 7 d after treatment. Means followed by the same letter are not significantly different ($P = 0.05$, Tukey's HSD test on arcsine transformed data).

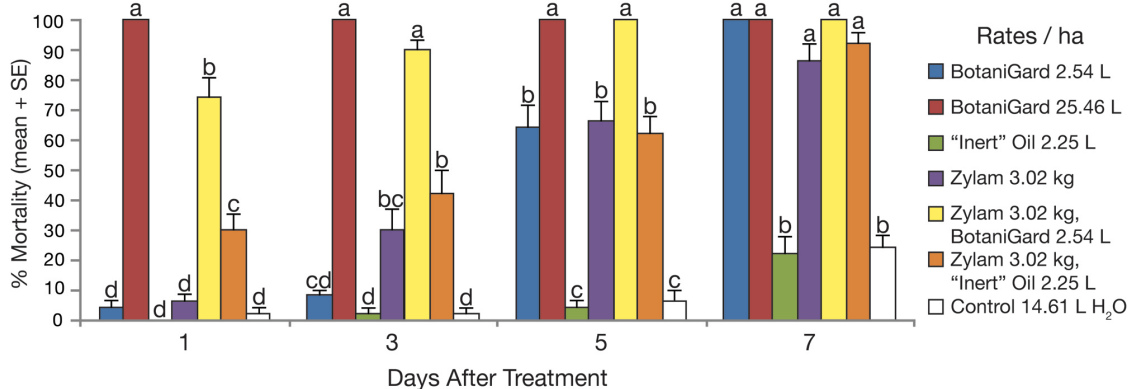


Figure 4: Percent mortality (mean + SE) of adult weevils to BotaniGard at one-tenth and the highest label dosage (2.54 and 25.4 liters/ha respectively), and label dosage of Zylam (dinotefuran) with and without one-tenth the label dosage of BotaniGard and its carrier oil in Petri dish assays 1, 3, 5 and 7 d after treatment. Means followed by the same letter are not significantly different ($P = 0.05$, Tukey's HSD test on arcsine transformed data).

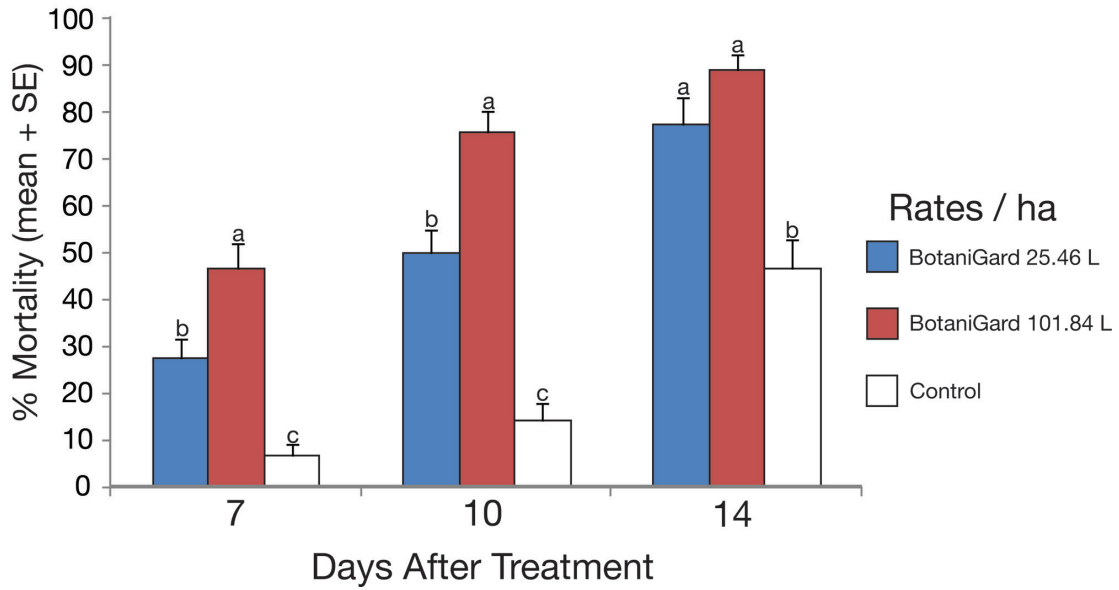


Figure 5: Percent mortality (mean + SE) of adults at the highest BotaniGard label dosage (25.46 liters/ha), 4× the highest label dosage (101.84 liters/ha) versus a water control in turfgrass plug assays 7, 10 and 14 d after treatment. Means followed by the same letter are not significantly different ($P = 0.05$, Tukey’s HSD test on arcsine transformed data).

CHAPTER 2

**“Determining the Amounts of Clothianidin and Imidacloprid in *Poa annua* (L.) by
ELISA and Their Effects With and Without Bifenthrin on *Listronotus maculicollis*
(Coleoptera: Curculionidae)”**

by

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ABSTRACT

ELISA plates were used to determine the amounts of clothianidin and imidacloprid in *Poa annua* clippings from treated golf course fairways. Amounts of clothianidin ranged from 71.8 to 1238.1 ng/g tissue in 2011 and 68.8 to 2045.0 ng/g tissue in 2012. Amounts of imidacloprid ranged from 40.8 to 1679.2 ng/g tissue in 2011 and 116.8 to 3722.0 ng/g tissue in 2012. *Listronotus maculicollis* adults were caged on neonicotinoid and neonicotinoid/pyrethroid treated *P. annua* plugs. Substantial feeding on *P. annua* was observed; however, mortality of *L. maculicollis* adults was not significantly different from control plugs. We were not able to determine concentrations of either clothianidin or imidacloprid that were effective in controlling larvae. Our data help to explain the lack of control of this insecticide resistant pest.

Key Words: ELISA, clothianidin, imidacloprid, bifenthrin, *Listronotus maculicollis*, Curculionidae

INTRODUCTION

Larvae of *Listronotus maculicollis* (Kirby) (Coleoptera: Curculionidae) are the most destructive insect pests of *Poa annua* L. (Poales: Poaceae) on golf courses in the northeastern United States (Vittum et al. 1999). This insect species was first seen damaging turfgrass in Connecticut in 1931 and by the late 1950s and early 1960s it was responsible for severe damage on golf courses in the state (Britton 1932, Tashiro 1976). Adult *L. maculicollis* chew notches on grass blades at the juncture of leaves and stems. Adult damage is not as severe as larval feeding, which can result in extensive turf damage and death since they feed at the plant crown. Where larval densities exceed 450 per 929 cm² (1 foot²), injury to golf course greens, collars, and fairways is common (Watschke et al. 1994). Instars 1-3 feed inside plant stems while 4th and 5th instars feed on plant crowns. There are normally two to three generations of *L. maculicollis* per year in the northeastern U. S.

Fourth generation pyrethroids provided excellent control of weevils in the 1990s and early 2000s. In 2005, the first indications of diminished pyrethroid effectiveness were reported (Vittum 2005). In 2009 the first study to confirm pyrethroid resistance was published (Ramoutar et al. 2009a). Two subsequent studies (Ramoutar et al. 2009b; 2010) further confirmed pyrethroid resistance.

We tested the hypothesis that treating *P. annua* plants early in the season would have enough neonicotinoid insecticide (either clothianidin or imidacloprid) in their tissue to control overwintering adult *L. maculicollis* as they feed. A second goal was to

determine the concentrations of clothianidin and imidacloprid in *P. annua* tissue necessary to kill larvae and how long these toxic levels remained in plant tissue.

METHODS

Fairway and tee treatments, *P. annua* tissue sampling, and turf plug assays with adults 2011. Two golf course tees and one fairway in Narragansett, RI were treated on 13 May 2011 with one of the following: 1.4 liters/ha Merit 2F (336 g imidacloprid) (Bayer Environmental Science, Research Triangle Park, NC), 4.44 liters/ha of Allectus SC (96 g bifenthrin, 239 g imidacloprid) (Bayer Environmental Science) or 0.85 liters/ha Aloft SC (112 g bifenthrin, 226 g clothianidin) (Arysta LifeScience, Cary, NC). *P. annua* clippings from treated tee and fairways were collected 7 d after initial treatment and periodically (normally weekly) up to 77 d and kept at -20°C. Twelve turf plugs were taken 4, 7, 11, and 14 d after treatment and brought back to the laboratory and placed in 148 ml plastic cups. Five to ten adult weevils were placed on plugs and the cups were covered with screening held in place by a rubber band. Control plugs were taken from an untreated *P. annua* plot established on the University of Rhode Island turfgrass research farm. Plugs were watered as needed and checked for adult mortality 7, 10, 14, 21 and 27 d after being placed on plugs.

Four golf course fairways in Westerly, RI were treated on 3 May 2011 with one of the following: 0.44 or 0.89 kg/ha of Arena 50WDG (224 g or 448 g clothianidin) (Valent USA Corp, Walnut Creek, CA) or with 0.85 or 1.05 liters/ha of Aloft SC (112 g bifenthrin, 226 g clothianidin or 138 g bifenthrin, 280 g clothianidin) (Arysta LifeScience Cary, NC). Two fairways which received the 0.44 kg/ha treatment of Arena and the 0.85 liters/ha of Aloft were treated a second time with the same rates as

the first applications on 20 June 2011. *P. annua* clippings from treated fairways were collected 7-8 d after initial treatment and periodically (normally weekly) up to 77 d and kept at -20°C. Sixteen turf plugs (5.72 cm diam.) were collected from treated fairways 8 and 15 d after the initial treatments and 8 d after the second treatments and treated as per above.

Fairway treatments, tissue and larval sampling 2012. Four replicates (37.16 sq. m) of the following treatments: 1.05 liters/ha Aloft SC (138 g bifenthrin, 280 g clothianidin), 0.89 kg/ha Arena 50WDG (448 g clothianidin), 5.26 liters/ha of Allectus SC, and 1.87 liters/ha Merit 2F were applied on April 17, 2012 to a golf course fairway in Baltic, CT using CO₂ sprayers and four nozzle wands equipped with 8003VS TeeJet nozzles (Spraying Systems Co., Wheaton, IL). Four replicates of the same treatments were applied to a golf course fairway in Westerly, RI on April 19, 2012.

Larval and *P. annua* tissue sampling began one week after application of insecticide treatments and continued weekly for 25 weeks. A 0.94 m × 1.52 m wood frame was placed within each plot leaving at least 1.5 m border to the perimeter of each plot. A grid pattern of strings were attached to the frame to make 230 (5.72 cm × 5.72 cm) squares equal to the diameter (5.72 cm) of the turf plug extractor. Flags were placed in squares to mark where turf plug samples would be taken and prevent resampling from the same location for the remainder of the season. At both sites (Westerly, RI; Baltic, CT), three turf plug samples were taken from each plot weekly. Two turf plugs from each plot were placed in modified Berlese funnels similar to the method used by Diaz (2008) (Fig. 1). Five milliliters of glycerin was used in the bottom collection containers to hold larvae. Funnel containers were checked up to 14 d after

collection and larvae were removed and placed in vials of 70% ethyl alcohol until head capsule width could be measured. Head capsule width was measured using a binocular microscope fitted with an eyepiece reticle at 63× magnification to determine larval instars. The third plug from each plot was used for collection of grass clippings used for ELISA determination of neonicotinoid concentration. At least 0.5 g (fresh weight) of grass clippings was cut from the turf plug then placed in a labeled plastic bag and stored at -20°C until analysis.

ELISA methods: Individual grass clippings were weighed (0.5 g per sample) and added to a 15-ml centrifuge tube containing 5 ml of methanol. The samples were gently shaken overnight at room temperature. Insecticide concentrations were quantified using commercially available ELISA kits (QuantiPlate[®] kit for imidacloprid available from EnviroLogix, 500 Riverside Industrial Parkway, Portland, ME; Smart Assay ELISA for clothianidin available from Horiba, 1761 Armstrong Ave, Irvine, CA). An aliquot (10 µl) of each extract was dried completely in a TurboVap[®] LV evaporator (Caliper Life Sciences, Hopkinton, MA, USA) and then reconstituted in a 0.05% aqueous solution of Triton X-100 prior to analysis by ELISA. The reconstituted samples were used directly for ELISA and were further diluted with 0.05% Triton X-100 to bring the concentrations of insecticide within the appropriate range of the standard curve for each ELISA test (0.2 – 6 ng ml⁻¹ imidacloprid and 1.5 – 15 ng ml⁻¹ clothianidin). The final concentrations of insecticide were converted to ng of insecticide per gram of plant tissue. A Molecular Devices SpectraMAX 250 microplate reader (Sunnyvale, CA) was used to read plates.

Statistical Analysis. Data were analyzed by analysis of variance (ANOVA) followed by mean separation by Tukey's HSD test (SAS version 9.2).

RESULTS

Adult control. *L. maculicollis* adults from Norwich, CT were not controlled by feeding on *P. annua* tissue for 7 or 14 d ($F = 4.23$; $df = 3,9$; $P = 0.04$; $F = 8.00$; $df = 3,9$; $P = 0.01$, respectively) with a concentration of clothianidin of 221 ng/g or concentrations of imidacloprid of 1,011 or 1,910 ng/g (Fig. 2). The lack of effectiveness of either clothianidin or imidacloprid for controlling adults was consistent over seven different trials. Adults from Pawtucket, RI were not controlled by feeding on *P. annua* tissue for 7 or 19 d ($F = 0.87$; $df = 3,9$; $P = 0.48$; $F = 4.68$; $df = 3,9$; $P = 0.03$, respectively) with a concentration of clothianidin of 533 ng/g or concentrations of imidacloprid of 593 or 854 ng/g (Fig. 3). Adults from Pawtucket, RI were not controlled by feeding on *P. annua* tissue for 16 or 27 d ($F = 1.00$; $df = 4,12$; $P = 0.44$; $F = 0.73$; $df = 4,12$; $P = 0.59$, respectively) with concentrations of clothianidin of 550, 780, 1,207 or 1,238 ng/g (Fig. 4). Adults from Westerly, RI were not controlled by feeding on *P. annua* tissue for 7 or 14 d ($F = 0.60$; $df = 4,12$; $P = 0.67$; $F = 2.73$; $df = 4,12$; $P = 0.07$, respectively) with concentrations of clothianidin of 94, 100, 783, or 1,136 ng/g (Fig. 5). Adults from Norwich, CT were not controlled by feeding on *P. annua* tissue for 7 or 14 d ($F = 1.60$; $df = 3,9$; $P = 0.25$; $F = 0.10$; $df = 3,9$; $P = 0.95$, respectively) with a concentration of clothianidin of 72 ng/g or concentrations of imidacloprid of 1,144 or 1,524 ng/g (Fig. 6). Adults from Narragansett, RI were not controlled by feeding on *P. annua* tissue for 7 or 12 d ($F = 0.60$; $df = 3,9$; $P = 0.63$; $F = 0.58$; $df = 3,9$; $P = 0.64$, respectively) with a concentration of clothianidin of 403 ng/g

or concentrations of imidacloprid of 1,073 or 1,288 ng/g (Fig. 7). Adults from Pawtucket, RI were not controlled by feeding on *P. annua* tissue for 10 or 21 d ($F = 0.88$; $df = 4,12$; $P = 0.50$; $F = 0.94$; $df = 4,12$; $P = 0.47$, respectively) with concentrations of clothianidin of 423, 430, 739 or 814 ng/g (Fig. 8).

Larval control. Clothianidin did not last long enough in plots at Baltic, CT to show any significant control of either 1-3, 4-5 or 1-5 instar larvae (Figs. 9, 10, 11). Imidacloprid lasted longer at higher levels in plant tissue at Baltic, CT, however, there were no significant differences in treated versus control plots of 1-3, 4-5, or 1-5 instar larvae (Figs. 12, 13, 14). The same patterns were seen in plots treated in Westerly, RI (Figs. 15-20).

DISCUSSION

Adults are not killed by the concentrations of neonicotinoids we found in *P. annua* tissue. This does not support the hypothesis that superintendents should “arm” *P. annua* plants very early in the season with neonicotinoid insecticides to control adult weevils which are emerging from overwintering sites as they begin to feed. We expected to see more adult mortality from the combination products that contained bifenthrin. However, bifenthrin has a Koc value of 236,610 ml/g (Pesticide Properties Database, 2013), which means that bifenthrin is tightly adsorbed to organic matter and, once dried on *P. annua* tissue, it may not be available for control unless it is rewetted via irrigation and/or dew. Bifenthrin’s estimated Henry’s constant of 7.2×10^{-3} atm- m^3 /mole indicates that volatilization from moist soil surfaces may occur (Bifenthrin Technical Fact Sheet). Rewetting of *P. annua* tissue did not occur in our turf plug assay.

The long soil half-lives of clothianidin and imidacloprid, 545 and 191 d respectively (Pesticide Properties Database, 2013), also led to the hypothesis that if these materials were applied early in the season to control overwintering adults, the concentrations inside *P. annua* tissue would still be high enough later in the season to control first generation larvae. This does not appear to be the case. First through third instars feed inside *P. annua* stems while 4th and 5th instars feed on plant crowns. Koppenhofer et al. (2012) found that applications of clothianidin or imidacloprid between April 15 and May 3rd provided an average of 54 and 48% control respectively, whereas applications between May 18th and June 10 provided averages of 64 and 78% control respectively. It appears that we applied these products earlier than optimal timing to demonstrate any significant control. This information is important for managing this pest.

There were 4th and 5th instars present as early as 24 and 26 April (Figs. 10, 13, 16, 19) which may have been controlled by the bifenthrin in the Aloft and Allectus treatments. This is supported by the fact that larval densities were higher in the Arena treated plots despite the fact that the clothianidin levels were higher to begin with (treated with 448 g ai/hectare) versus larval densities in Aloft treated plots, which was treated with 280 g ai/hectare. The levels of clothianidin were also consistently higher in *P. annua* tissue in Arena versus Aloft treated plots. The same goes for the Merit and Allectus treated plots which were treated with (448 and 280 g ai/hectare respectively). The levels of imidacloprid were also consistently higher in *P. annua* tissue in Merit versus Allectus treated plots.

In 10 of 12 analyses larval counts were lower where bifenthrin was one of the treatment components (Figs. 9 – 20). Although some companies are guaranteeing season-long control, our data did not show any season-long control at either Baltic, CT (Fig. 21) or Westerly, RI (Fig. 22). Koppenhofer et al. (2012) analyzed data from 1,064 field experiments with various insecticides for annual bluegrass weevil control. Of these, 57 were Merit applications (various formulations) with imidacloprid rates between 140 and 560 g ai/ha. The majority of applications were with either 337 g ai/ha (32 applications) or 448 g ai/ha (14 applications). Four of the 337 g ai/ha applications showed zero percent control, even though they were applied when I would expect some level of control of larvae. Similarly, a total of 49 Arena applications (46 were 50 WDG and 3 were 0.5G formulations) with clothianidin rates between 168 and 449 g ai/ha were analyzed. The majority of these applications were either 224 g ai/ha (13 applications) or 449 g ai/ha (15 applications). Two of the 280 g ai/ha, one 337 g ai/ha, and one 449 g ai/ha applications showed zero percent control, again, when we would expect some level of control. Koppenhofer et al. (2012) found that several populations of *L. maculicollis* could be labeled as resistant to pyrethroids, organophosphates, neonicotinoids, indoxacarb, and bifenthrin / neonicotinoid combination products. If the populations of *L. maculicollis* at Baltic, CT and Westerly, RI were among those that demonstrated multiple resistance, this could explain the lack of control in our experiments.

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FOOTNOTES

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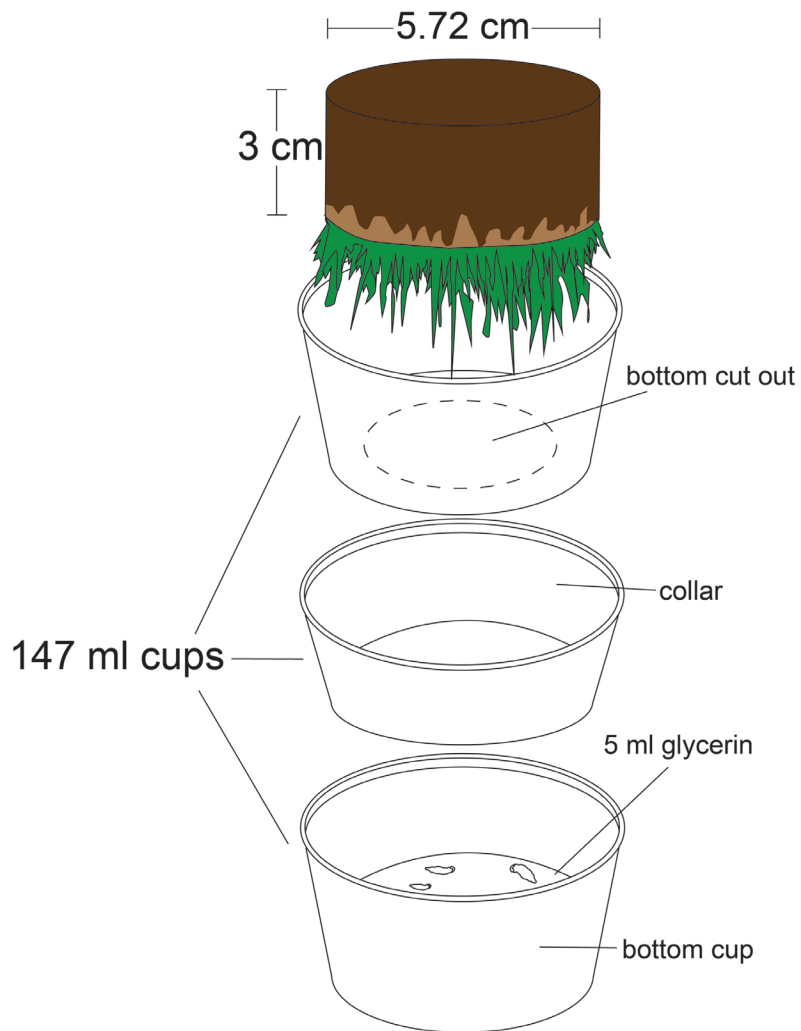


Fig. 1. Modified “Berlese funnel” system to collect larvae from *P. annua* turfgrass plugs.

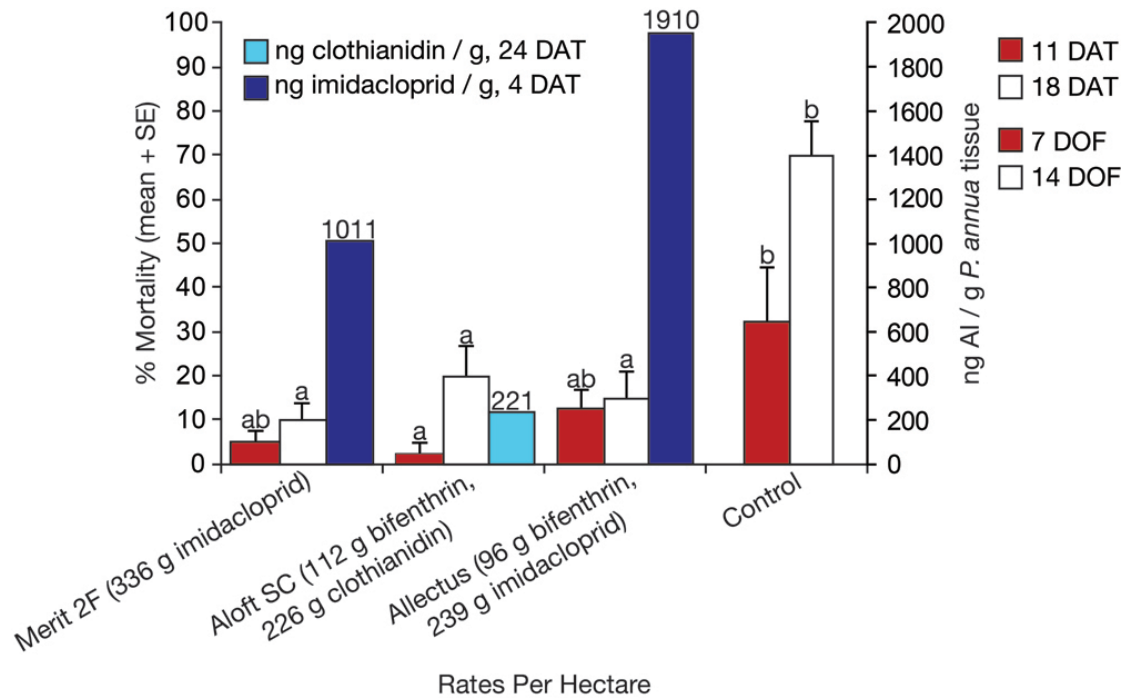


Fig. 2. Percent mortality (mean + SE) of adult weevils from Norwich, CT after feeding for 7 or 14 days on plugs of *P. annua* containing 1,011 or 1,910 ng imidacloprid or 221 ng clothianidin / g tissue. Means followed by the same letter are not significantly different ($P = 0.05$, Tukey's HSD test). DAT = days after treatment. DOF = days of feeding.

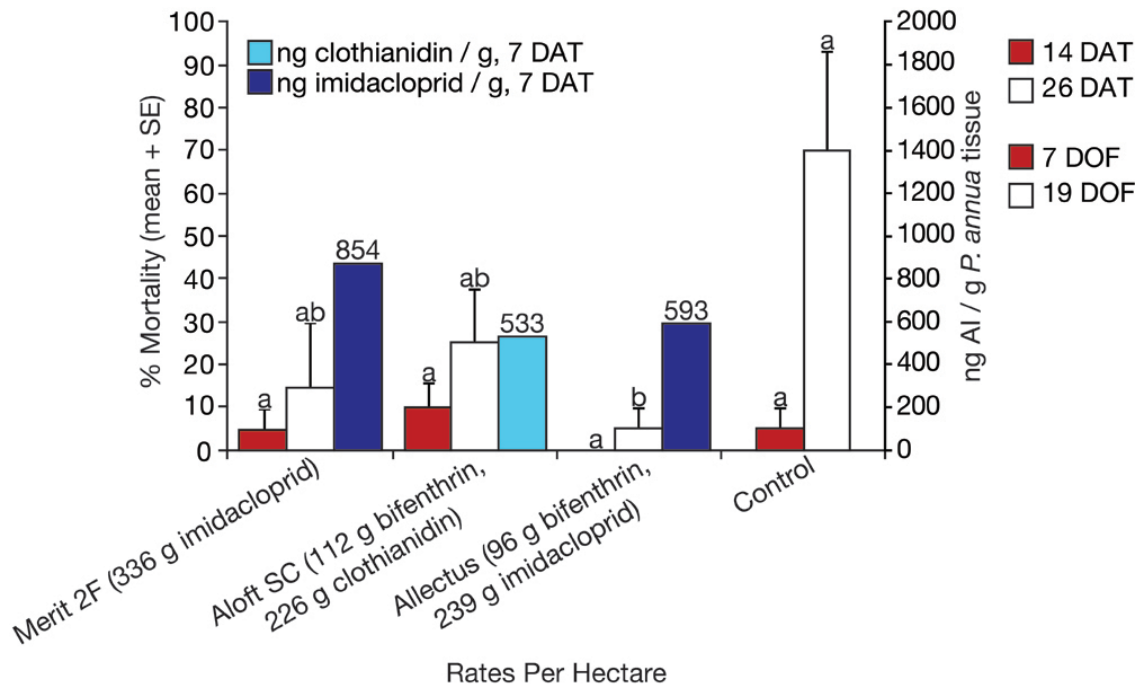


Fig. 3. Percent mortality (mean + SE) of adult weevils from Pawtucket, RI after feeding for 7 or 19 days on plugs of *P. annua* containing 593 or 854 ng imidacloprid or 533 ng clothianidin / g tissue. Means followed by the same letter are not significantly different ($P = 0.05$, Tukey's HSD test). DAT = days after treatment. DOF = days of feeding.

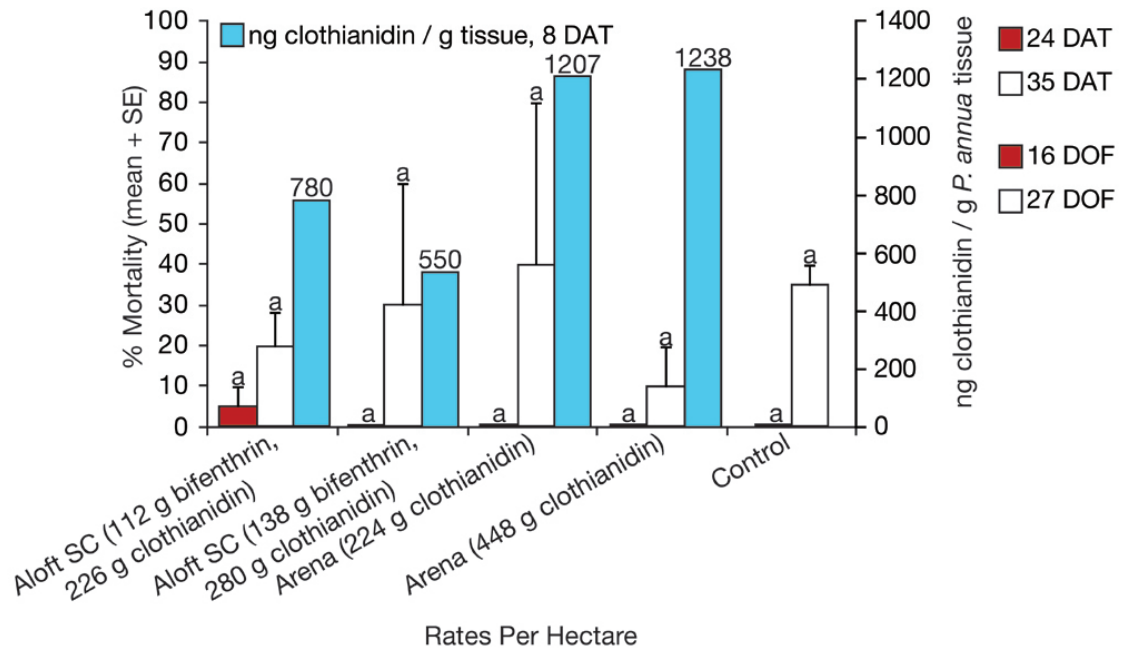


Fig. 4. Percent mortality (mean + SE) of adult weevils from Pawtucket, RI after feeding for 16 or 27 days on plugs of *P. annua* containing 550, 780, 1,207 or 1,238 ng clothianidin / g tissue. Means followed by the same letter are not significantly different ($P = 0.05$, Tukey's HSD test). DAT = days after treatment. DOF = days of feeding.

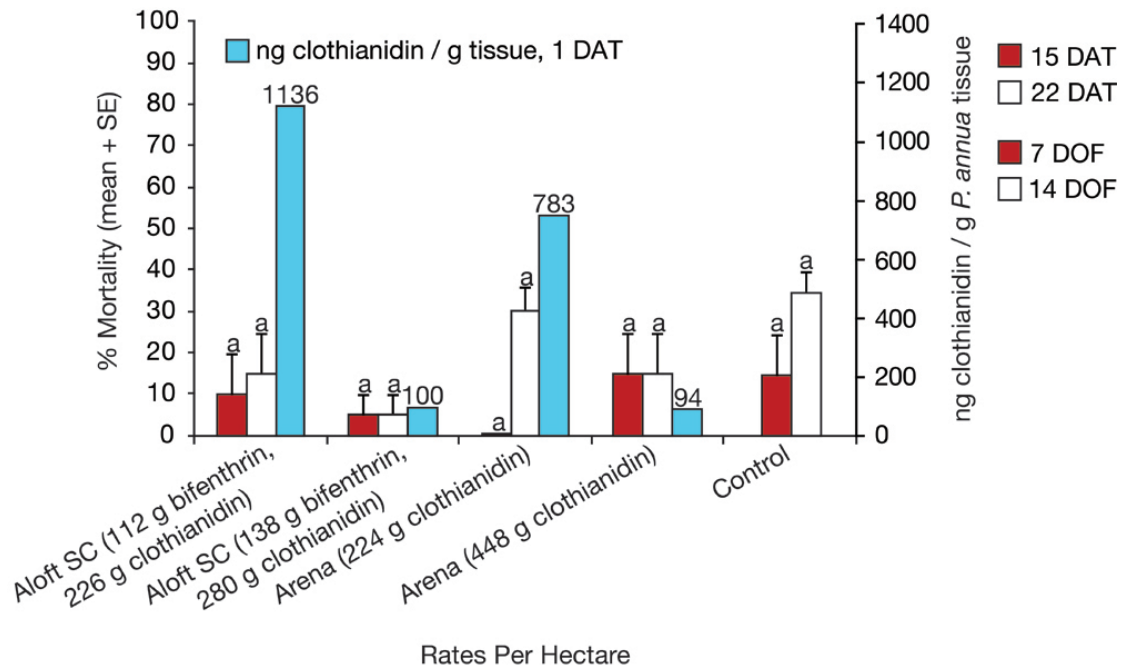


Fig. 5. Percent mortality (mean + SE) of adult weevils from Westerly, RI after feeding for 7 or 14 days on plugs of *P. annua* containing 94, 100, 783, or 1,136 ng clothianidin / g tissue. Means followed by the same letter are not significantly different ($P = 0.05$, Tukey's HSD test). DAT = days after treatment. DOF = days of feeding.

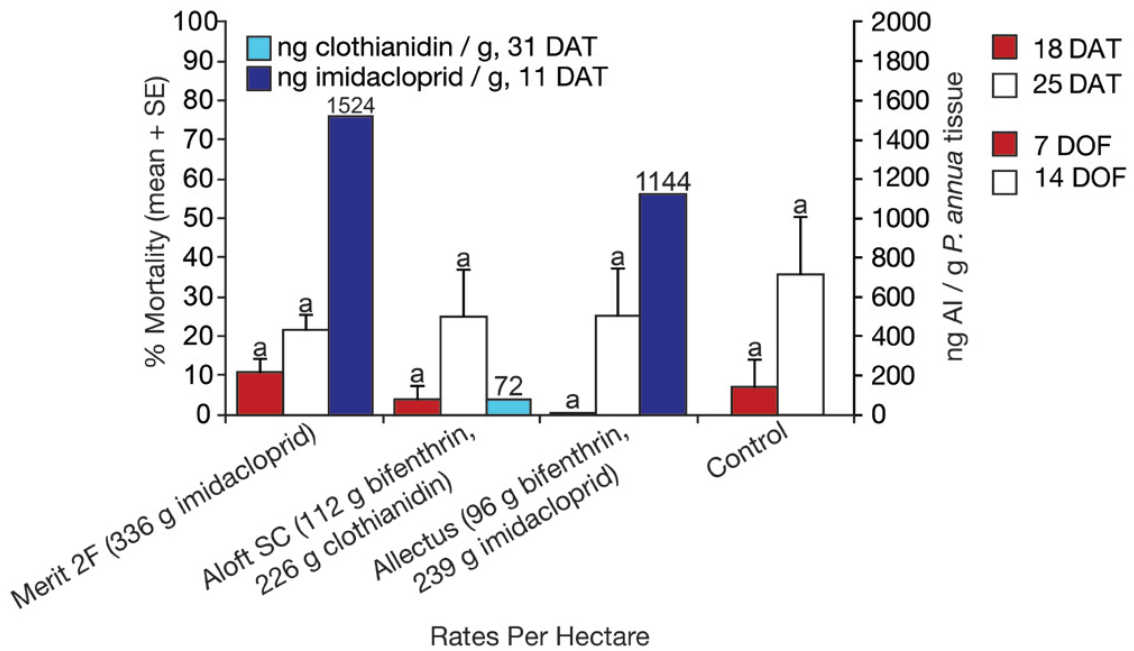


Fig. 6. Percent mortality (mean + SE) of adult weevils from Norwich, CT after feeding for 7 or 14 days on plugs of *P. annua* containing 1,144 or 1,524 ng imidacloprid or 72 ng clothianidin / g tissue. Means followed by the same letter are not significantly different ($P = 0.05$, Tukey's HSD test). DAT = days after treatment. DOF = days of feeding.

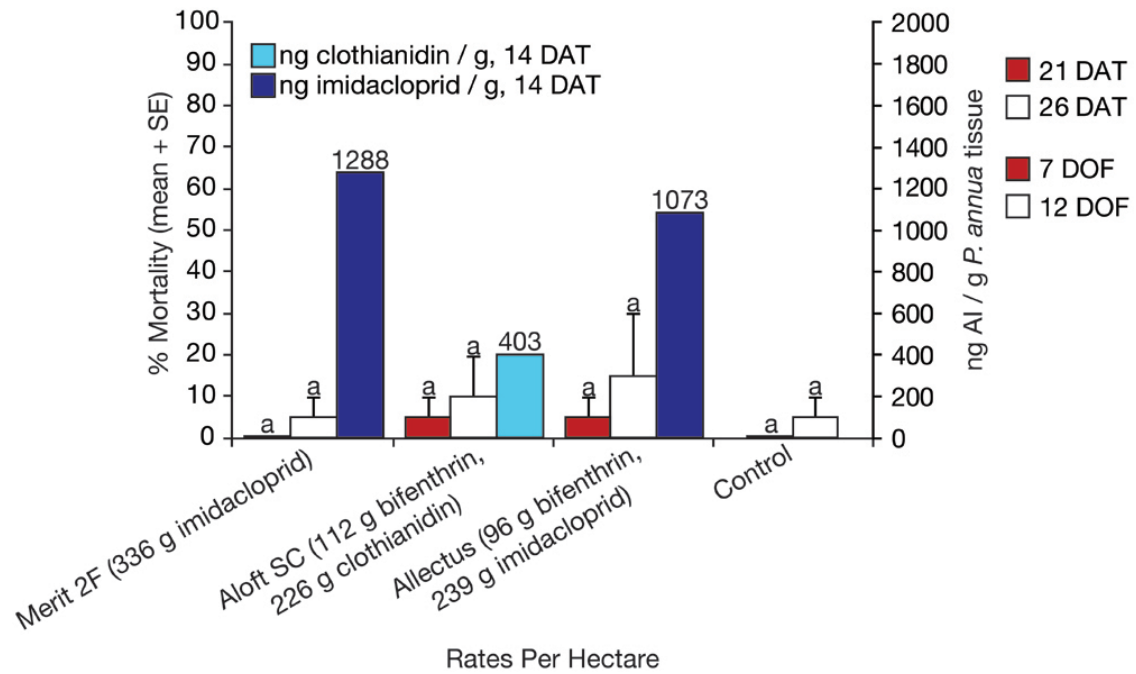


Fig. 7. Percent mortality (mean + SE) of adult weevils from Narragansett, RI after feeding for 7 or 12 days on plugs of *P. annua* containing 1,073 or 1,288 ng imidacloprid or 403 ng clothianidin / g tissue. Means followed by the same letter are not significantly different ($P = 0.05$, Tukey's HSD test). DAT = days after treatment. DOF = days of feeding.

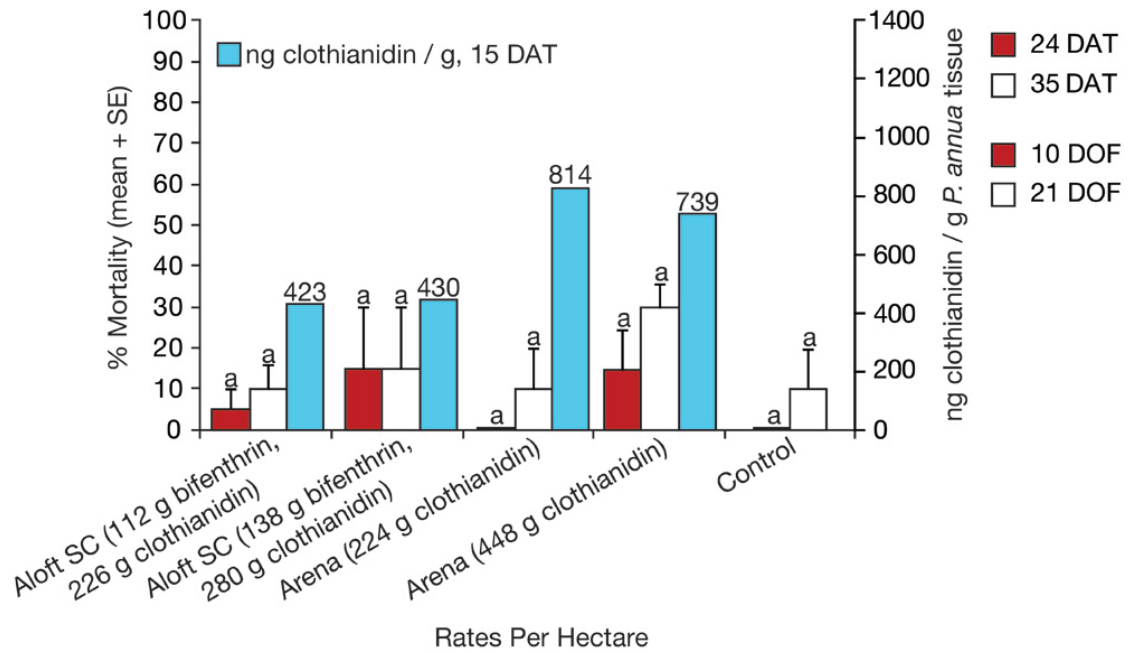


Fig. 8. Percent mortality (mean + SE) of adult weevils from Pawtucket, RI after feeding for 10 or 21 days on plugs of *P. annua* containing 423, 430, 739 or 814 ng clothianidin / g tissue. Means followed by the same letter are not significantly different ($P = 0.05$, Tukey's HSD test). DAT = days after treatment. DOF = days of feeding.

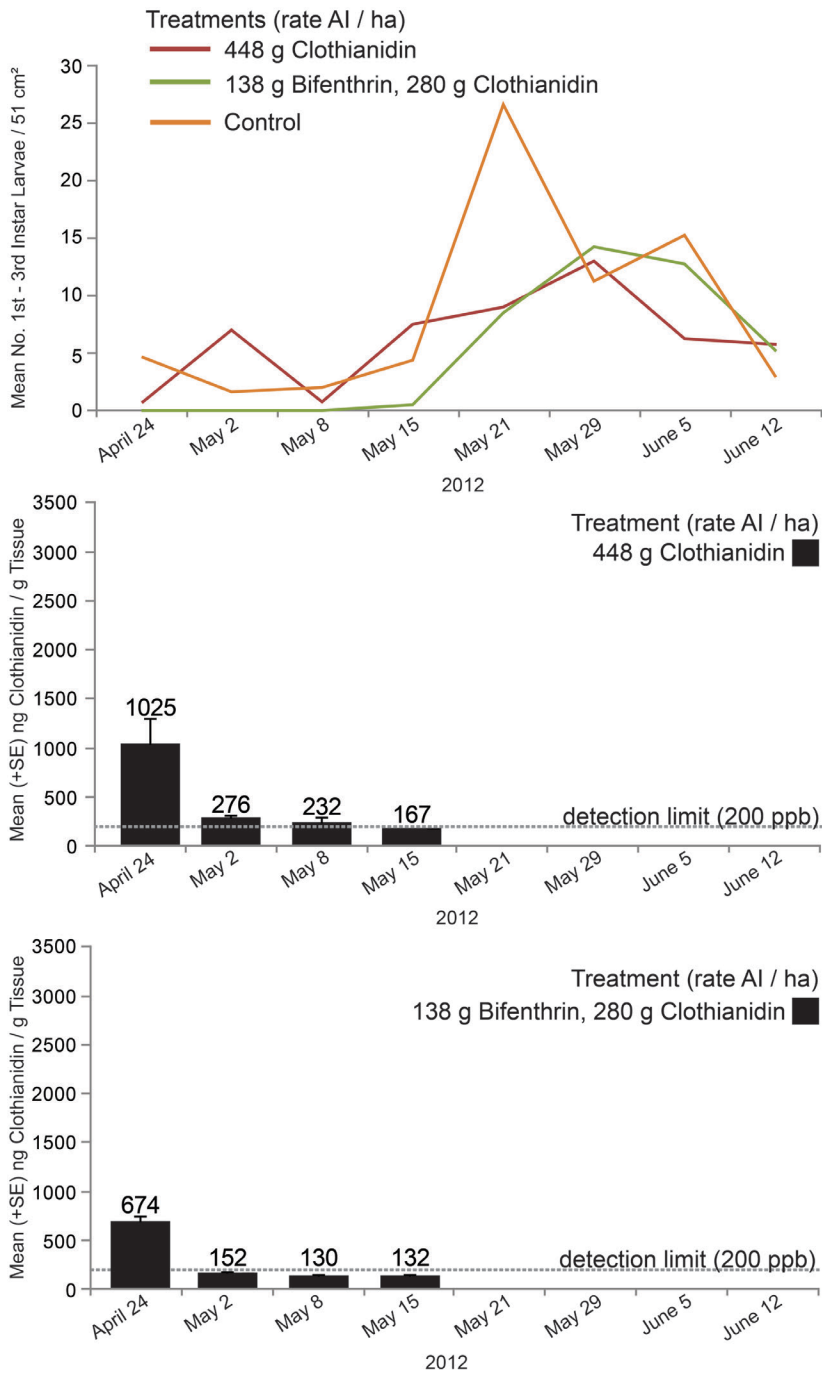


Fig. 9. Mean number of 1st-3rd instar larvae per 51 cm² of turfgrass and amounts of clothianidin in treatments where 0.89 kg/ha Arena 50WDG (448 g clothianidin) and 1.05 liters/ha Aloft SC (138 g bifenthrin / 280 g clothianidin) were applied on 17 April 2012 in Baltic, CT.

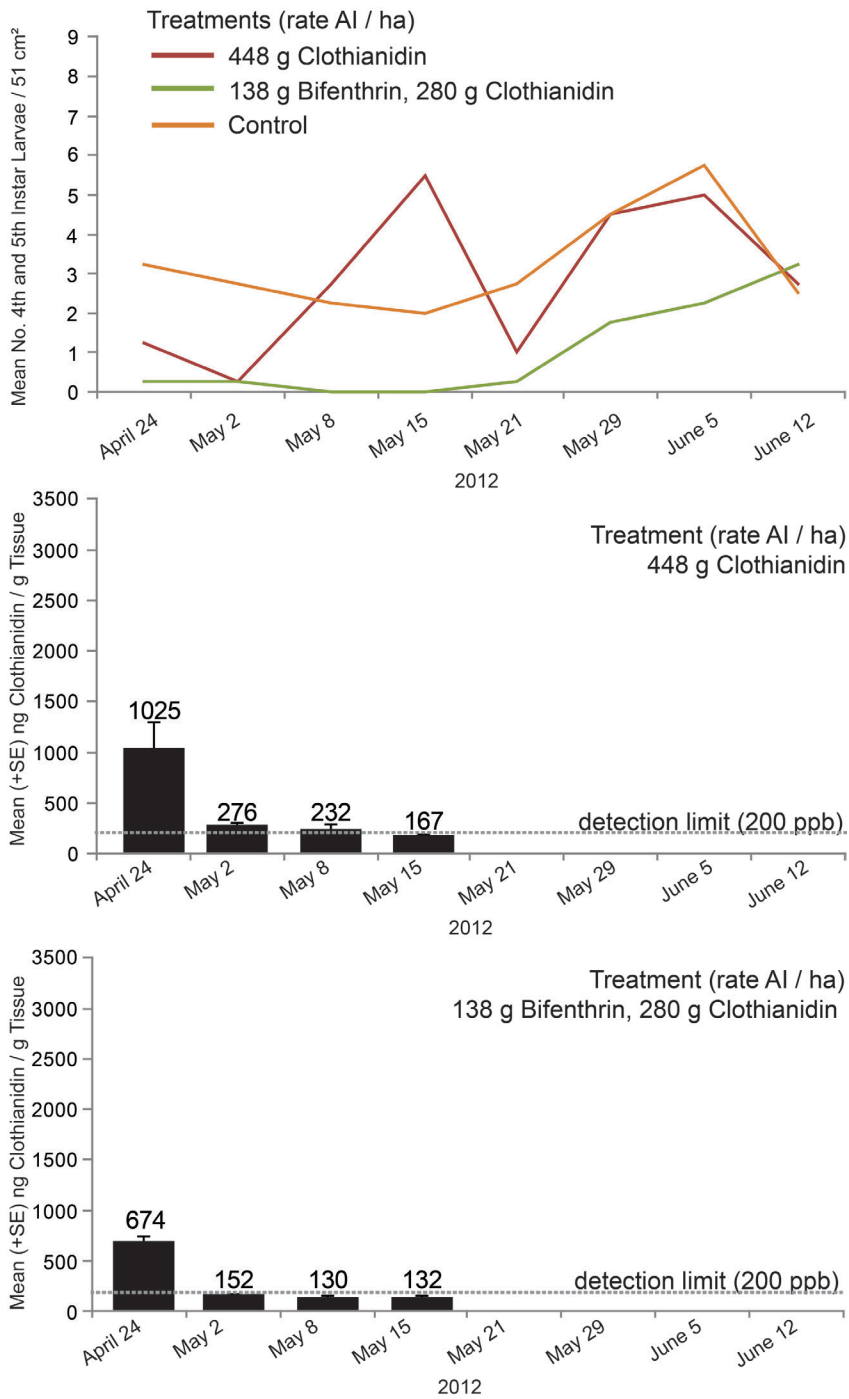


Fig. 10. Mean number of 4th-5th instar larvae per 51 cm² of turfgrass and amounts of clothianidin in treatments where 0.89 kg/ha Arena 50WDG (448 g clothianidin) and 1.05 liters/ha Aloft SC (138 g bifenthrin / 280 g clothianidin) were applied on 17 April 2012 in Baltic, CT.

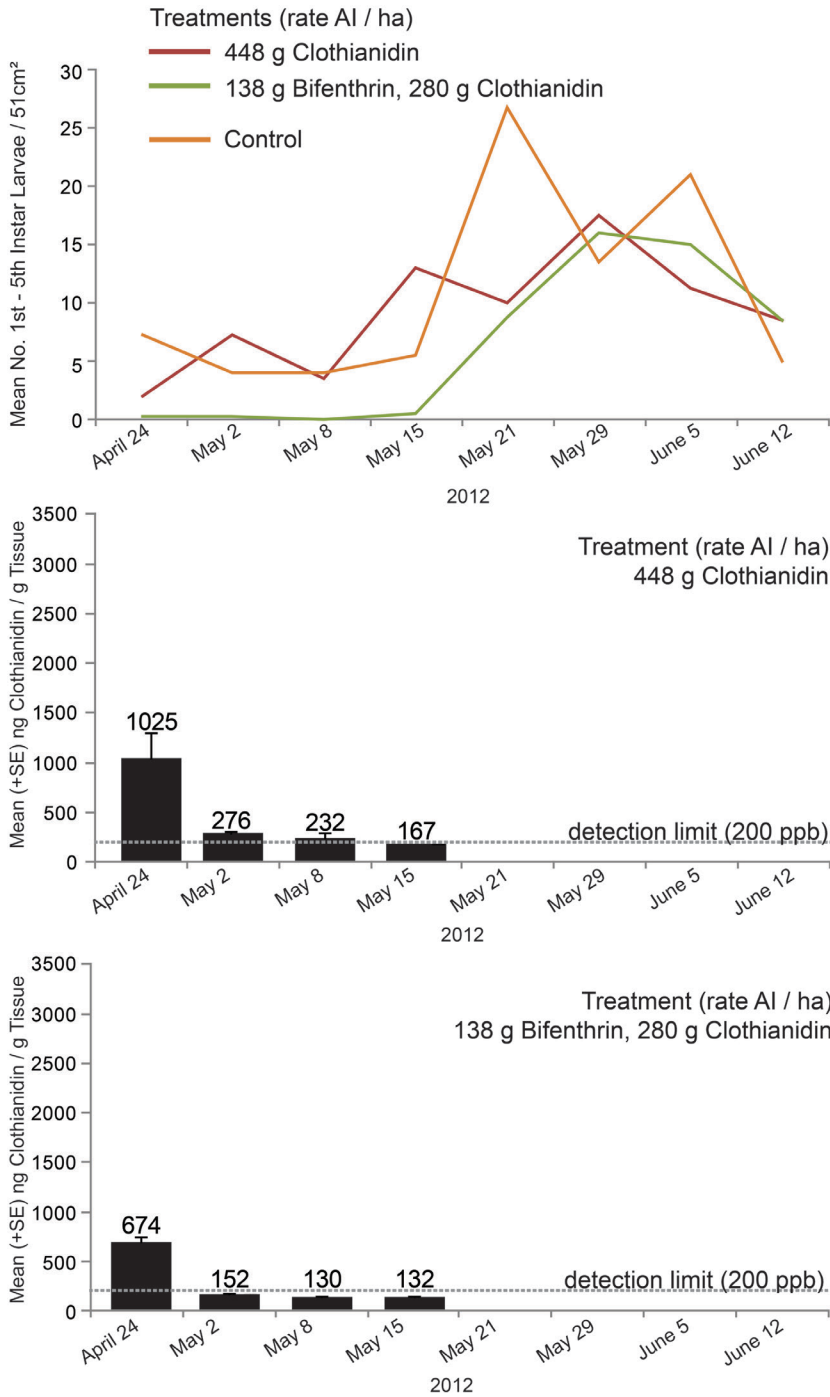


Fig. 11. Mean number of 1st-5th instar larvae per 51 cm² of turfgrass and amounts of clothianidin in treatments where 0.89 kg/ha Arena 50WDG (448 g clothianidin) and 1.05 liters/ha Aloft SC (138 g bifenthrin / 280 g clothianidin) were applied on 17 April 2012 in Baltic, CT.

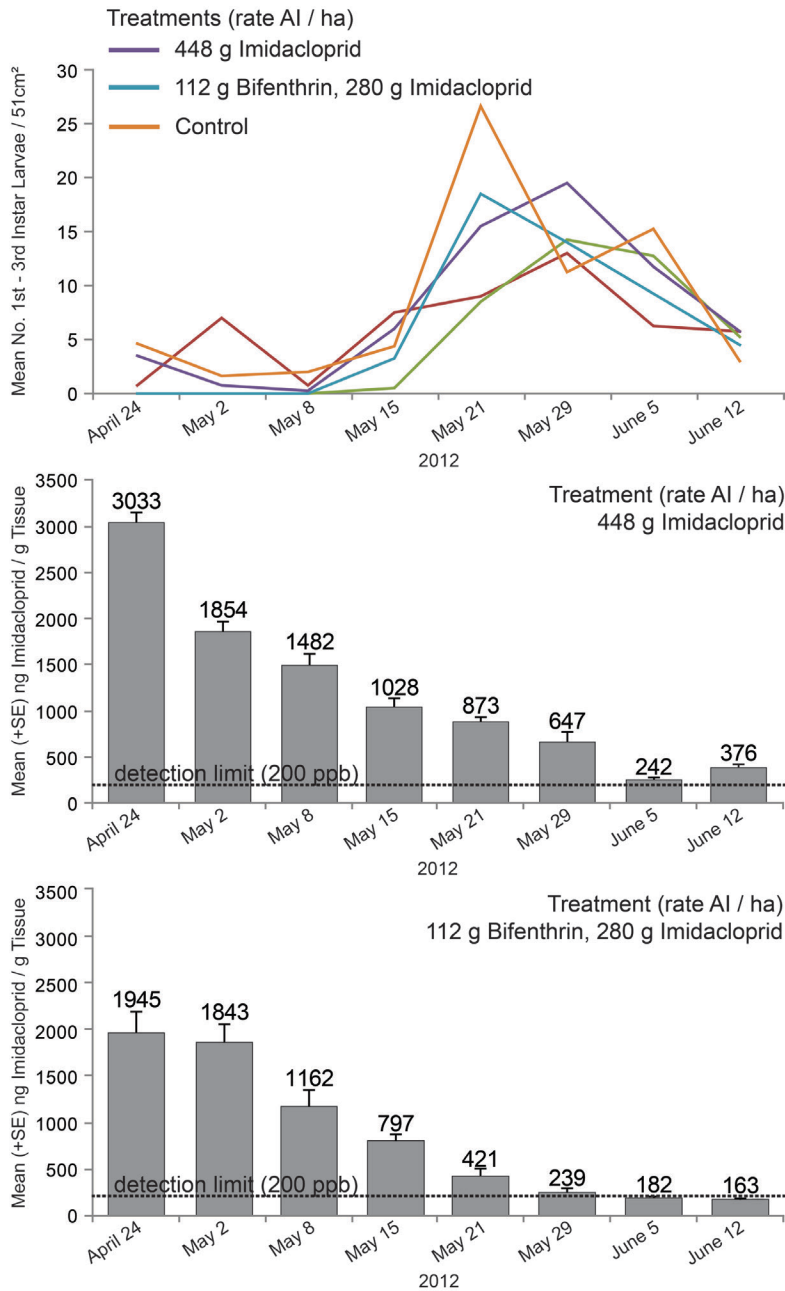


Fig. 12. Mean number of 1st-3rd instar larvae per 51 cm² of turfgrass and amounts of imidacloprid in treatments where 1.87 liters/ha Merit 2F (448 g imidacloprid) and 5.26 liters/ha Allectus SC (112 g bifenthrin / 280 g imidacloprid) were applied on 17 April 2012 in Baltic, CT.

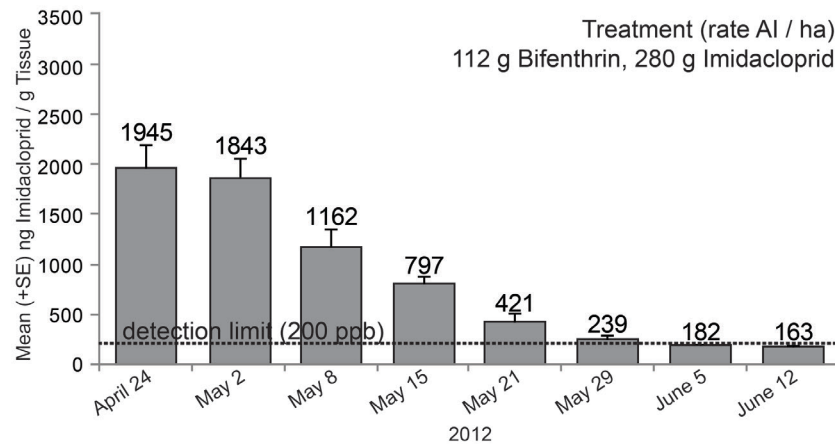
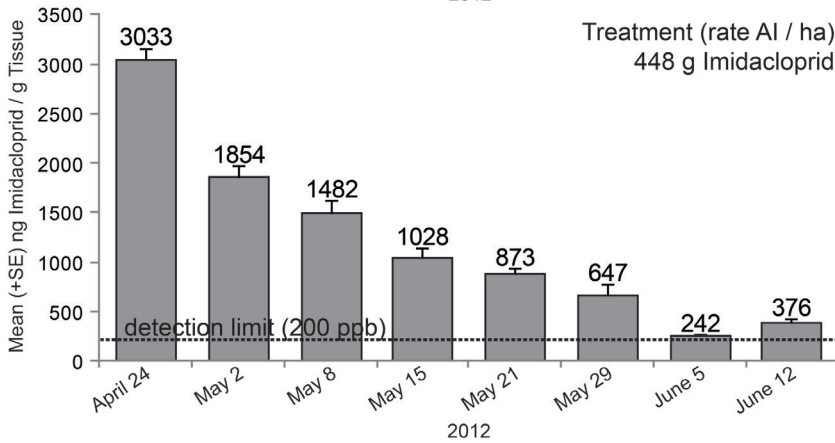
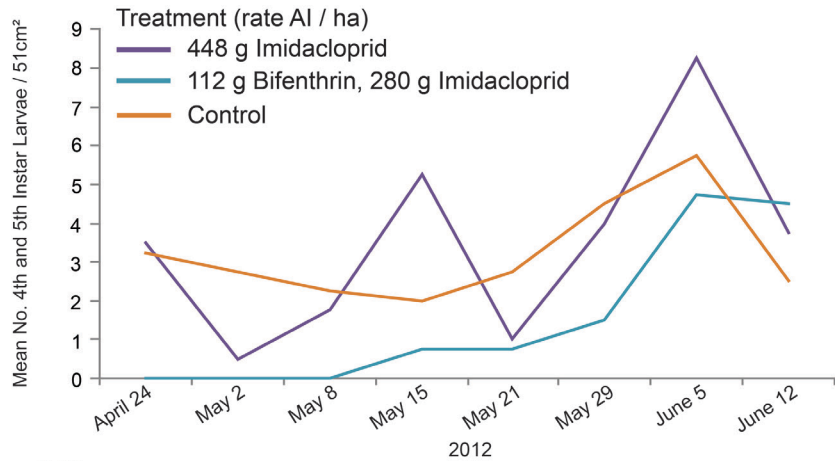


Fig. 13. Mean number of 4th-5th instar larvae per 51 cm² of turfgrass and amounts of imidacloprid in treatments where 1.87 liters/ha Merit 2F (448 g imidacloprid) and 5.26 liters/ha Allectus SC (112 g bifenthrin / 280 g imidacloprid) were applied on 17 April 2012 in Baltic, CT.

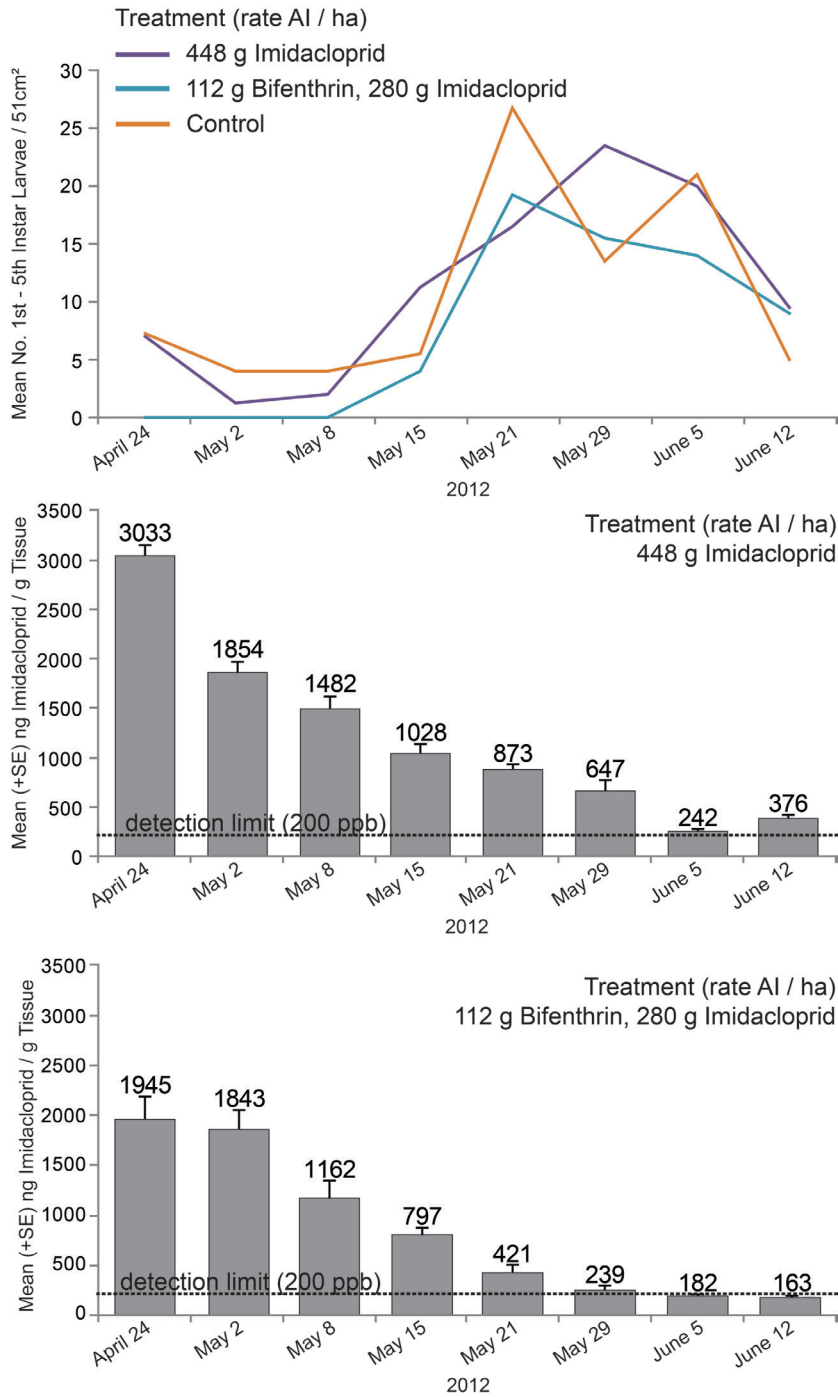


Fig. 14. Mean number of 1st-5th instar larvae per 51 cm² of turfgrass and amounts of imidacloprid in treatments where 1.87 liters/ha Merit 2F (448 g imidacloprid) and 5.26 liters/ha Allectus SC (112 g bifenthrin / 280 g imidacloprid) were applied on 17 April 2012 in Baltic, CT.

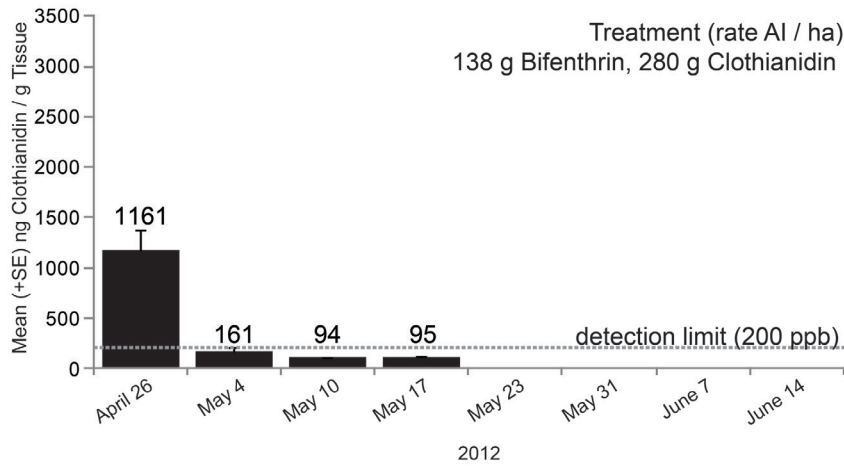
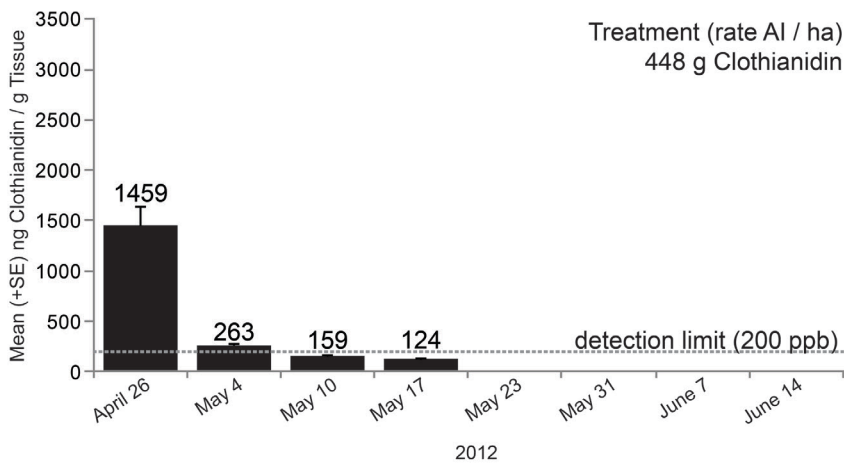
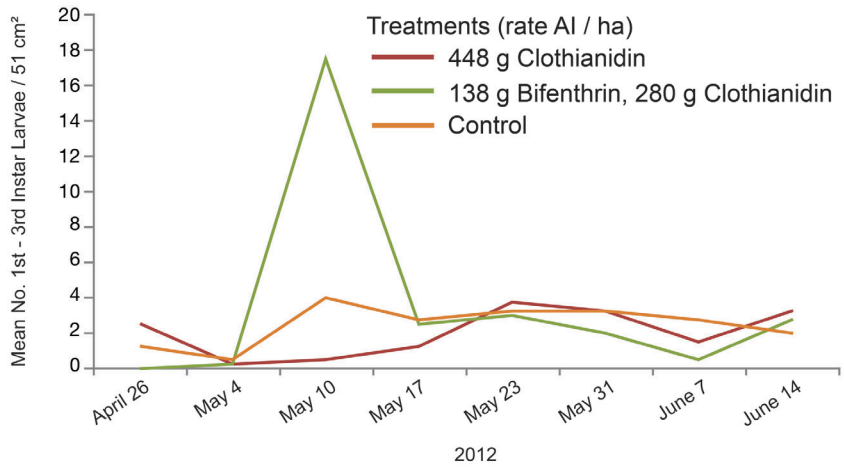


Fig. 15. Mean number of 1st-3rd instar larvae per 51 cm² of turfgrass and amounts of clothianidin in treatments where 0.89 kg/ha Arena 50WDG (448 g clothianidin) and 1.05 liters/ha Aloft SC (138 g bifenthrin / 280 g clothianidin) were applied on 19 April 2012 in Westerly, RI.

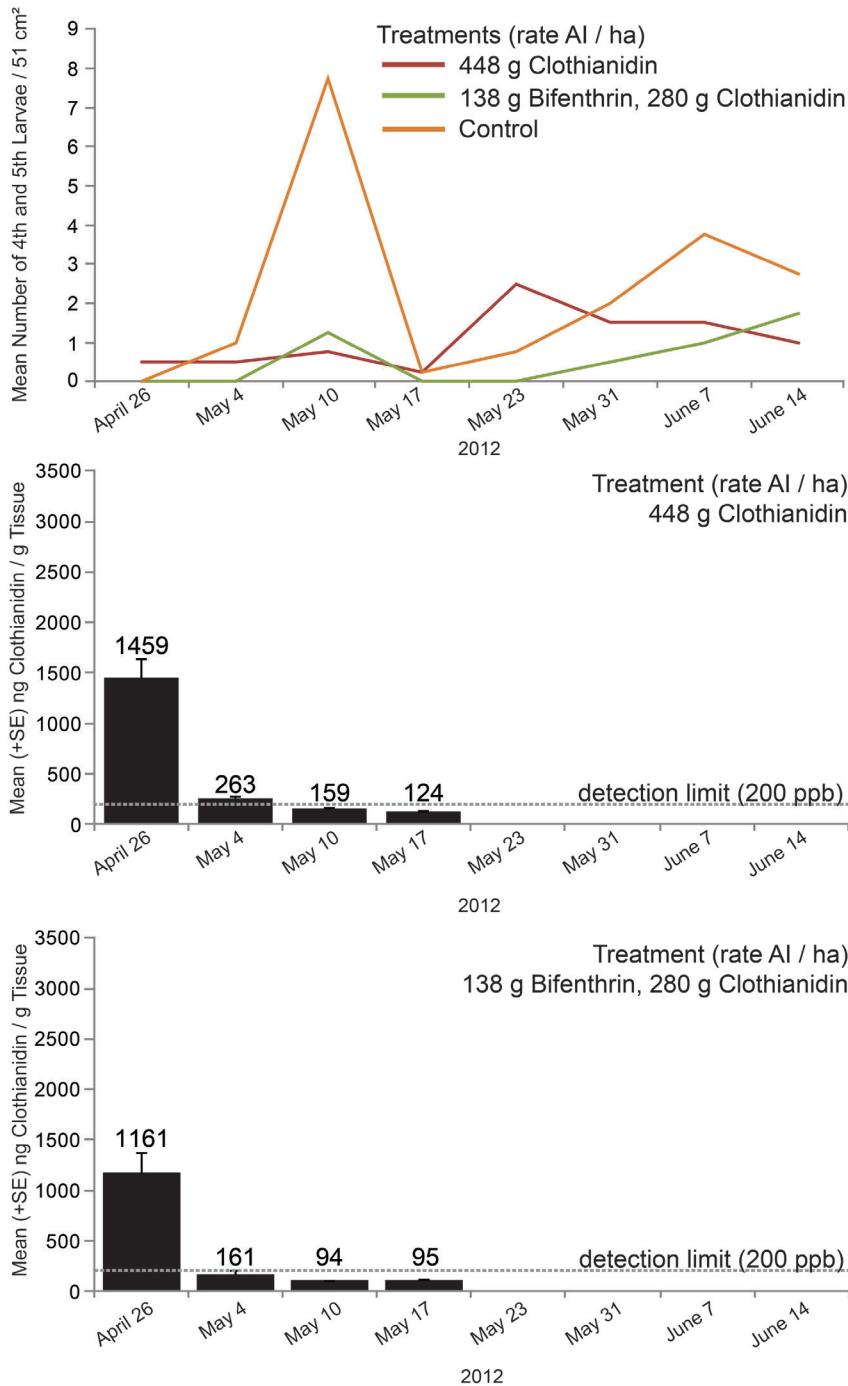


Fig. 16. Mean number of 4th-5th instar larvae per 51 cm² of turfgrass and amounts of clothianidin in treatments where 0.89 kg/ha Arena 50WDG (448 g clothianidin) and 1.05 liters/ha Aloft SC (138 g bifenthrin / 280 g clothianidin) were applied on 19 April 2012 in Westerly, RI.

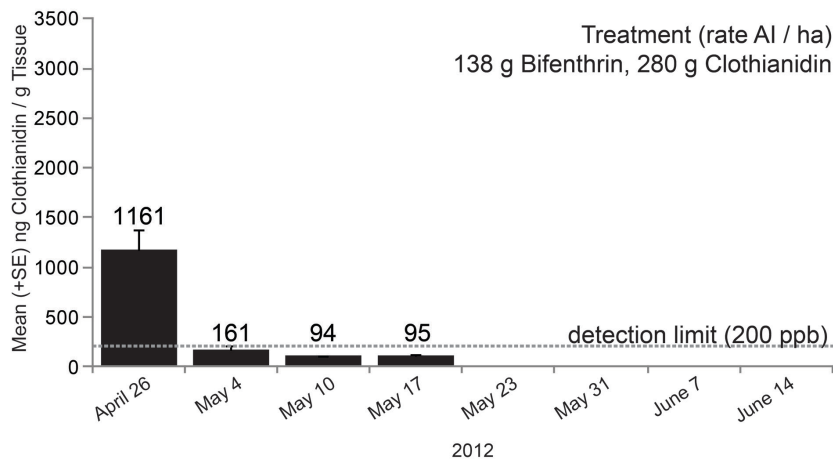
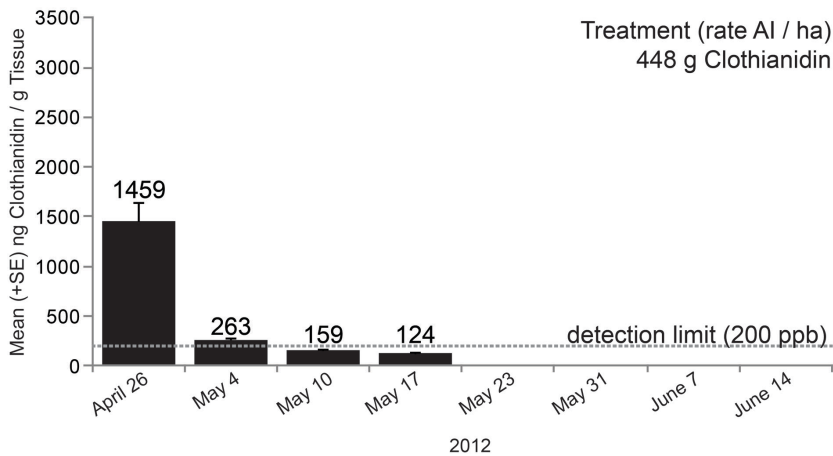
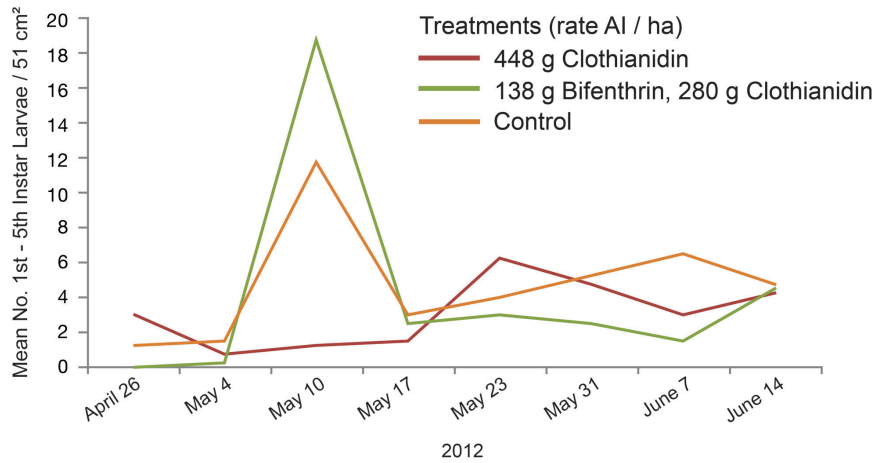


Fig. 17. Mean number of 1st-5th instar larvae per 51 cm² of turfgrass and amounts of clothianidin in treatments where 0.89 kg/ha Arena 50WDG (448 g clothianidin) and 1.05 liters/ha Aloft SC (138 g bifenthrin / 280 g clothianidin) were applied on 19 April 2012 in Westerly, RI.

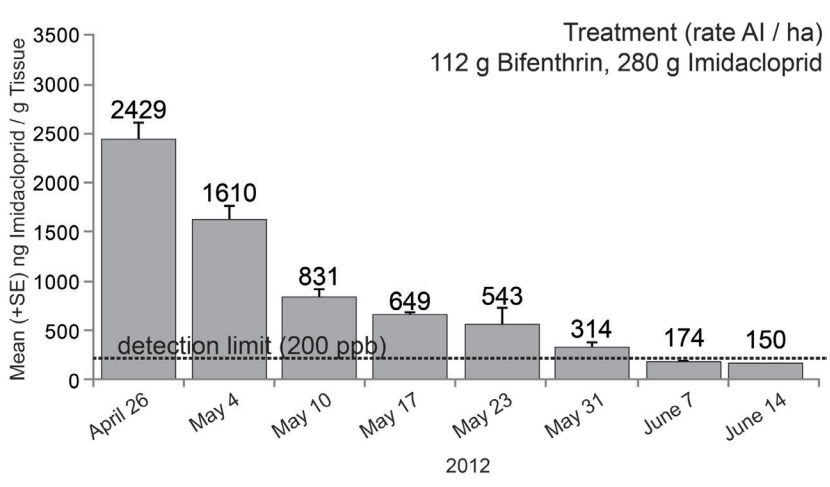
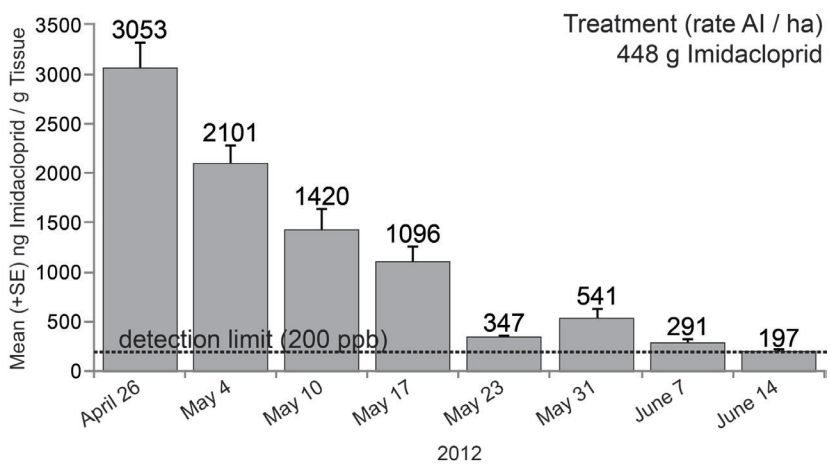
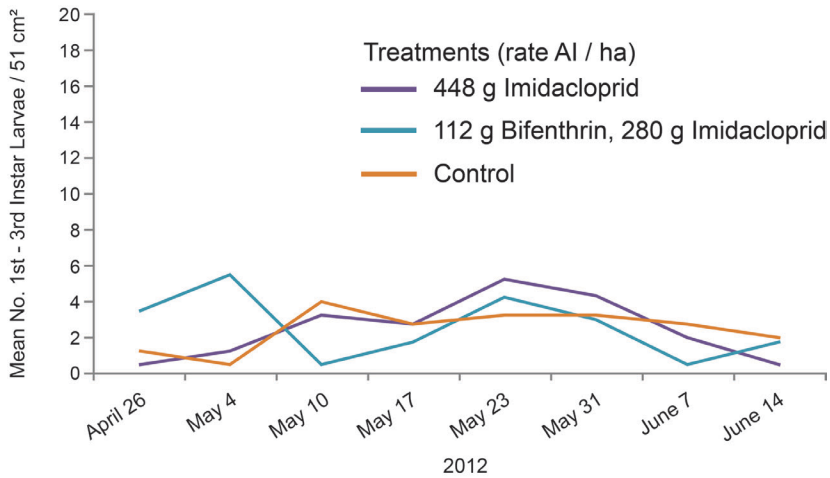


Fig. 18. Mean number of 1st-3rd instar larvae per 51 cm² of turfgrass and amounts of imidacloprid in treatments where 1.87 liters/ha Merit 2F (448 g imidacloprid) and 5.26 liters/ha Allectus SC (112 g bifenthrin / 280 g imidacloprid) were applied on 19 April 2012 in Westerly, RI.

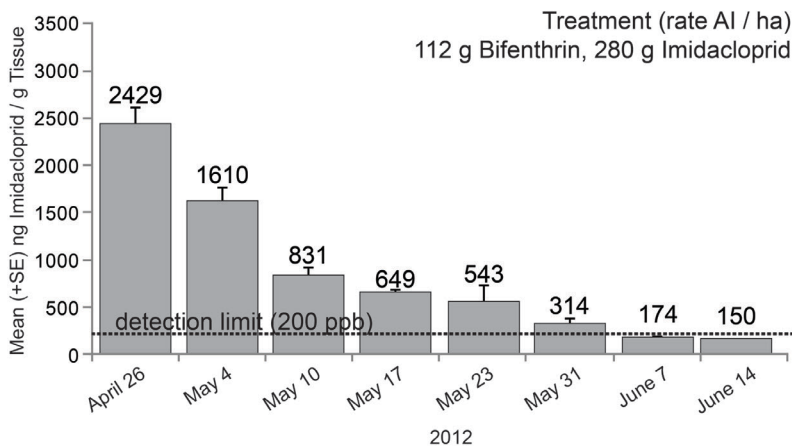
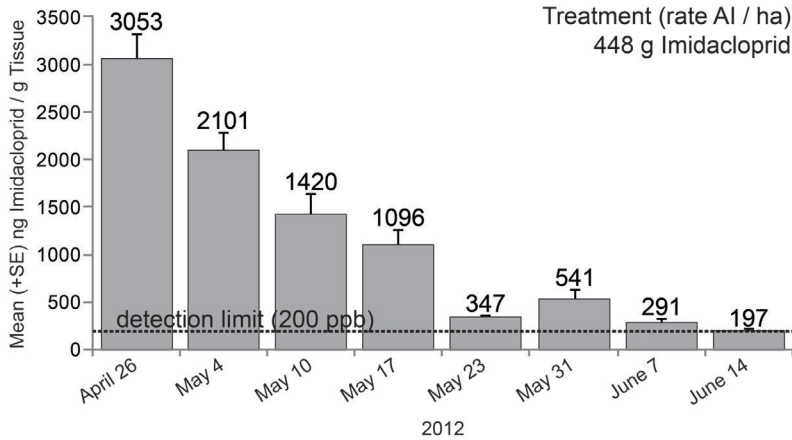
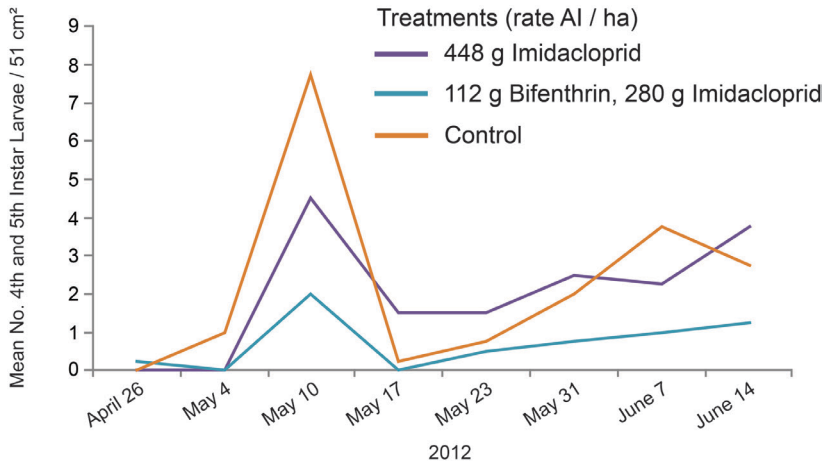


Fig. 19. Mean number of 4th-5th instar larvae per 51 cm² of turfgrass and amounts of imidacloprid in treatments where 1.87 liters/ha Merit 2F (448 g imidacloprid) and 5.26 liters/ha Allectus SC (112 g bifenthrin / 280 g imidacloprid) were applied on 19 April 2012 in Westerly, RI.

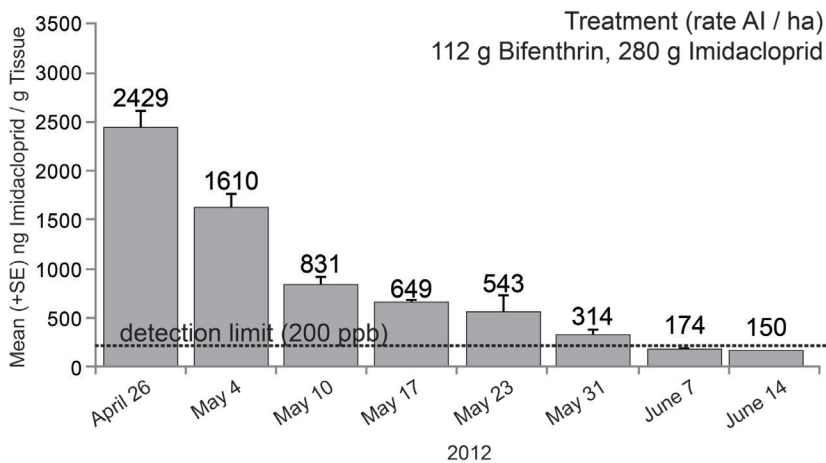
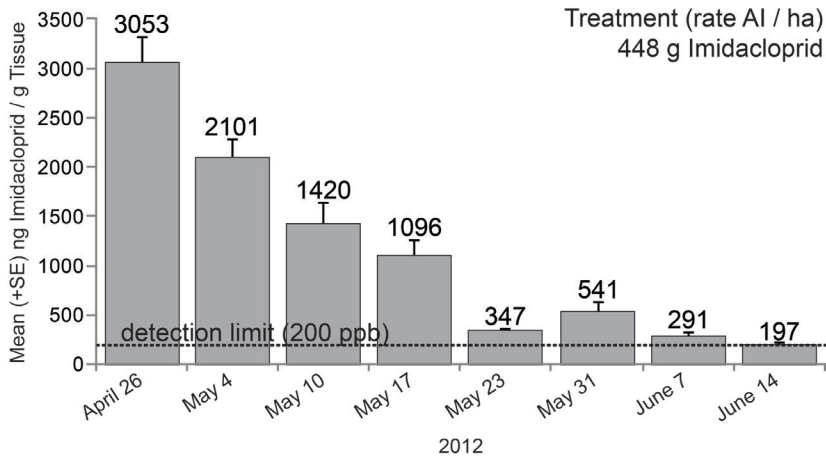
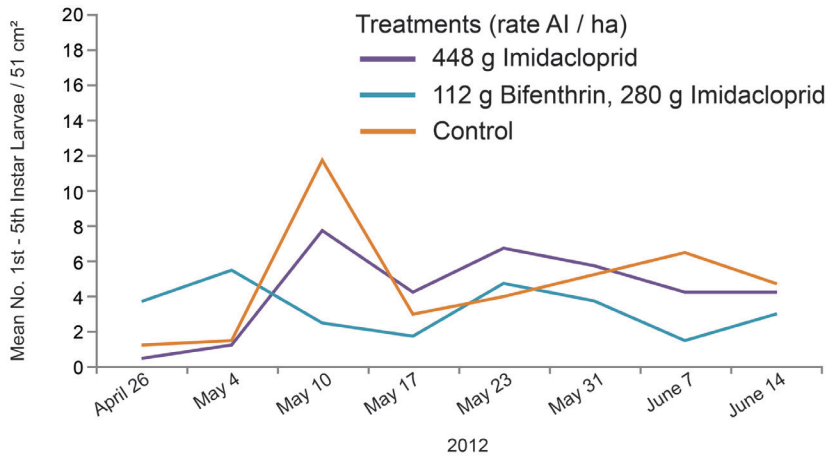


Fig. 20. Mean number of 1st-5th instar larvae per 51 cm² of turfgrass and amounts of imidacloprid in treatments where 1.87 liters/ha Merit 2F (448 g imidacloprid) and 5.26 liters/ha Allectus SC (112 g bifenthrin / 280 g imidacloprid) were applied on 19 April 2012 in Westerly, RI.

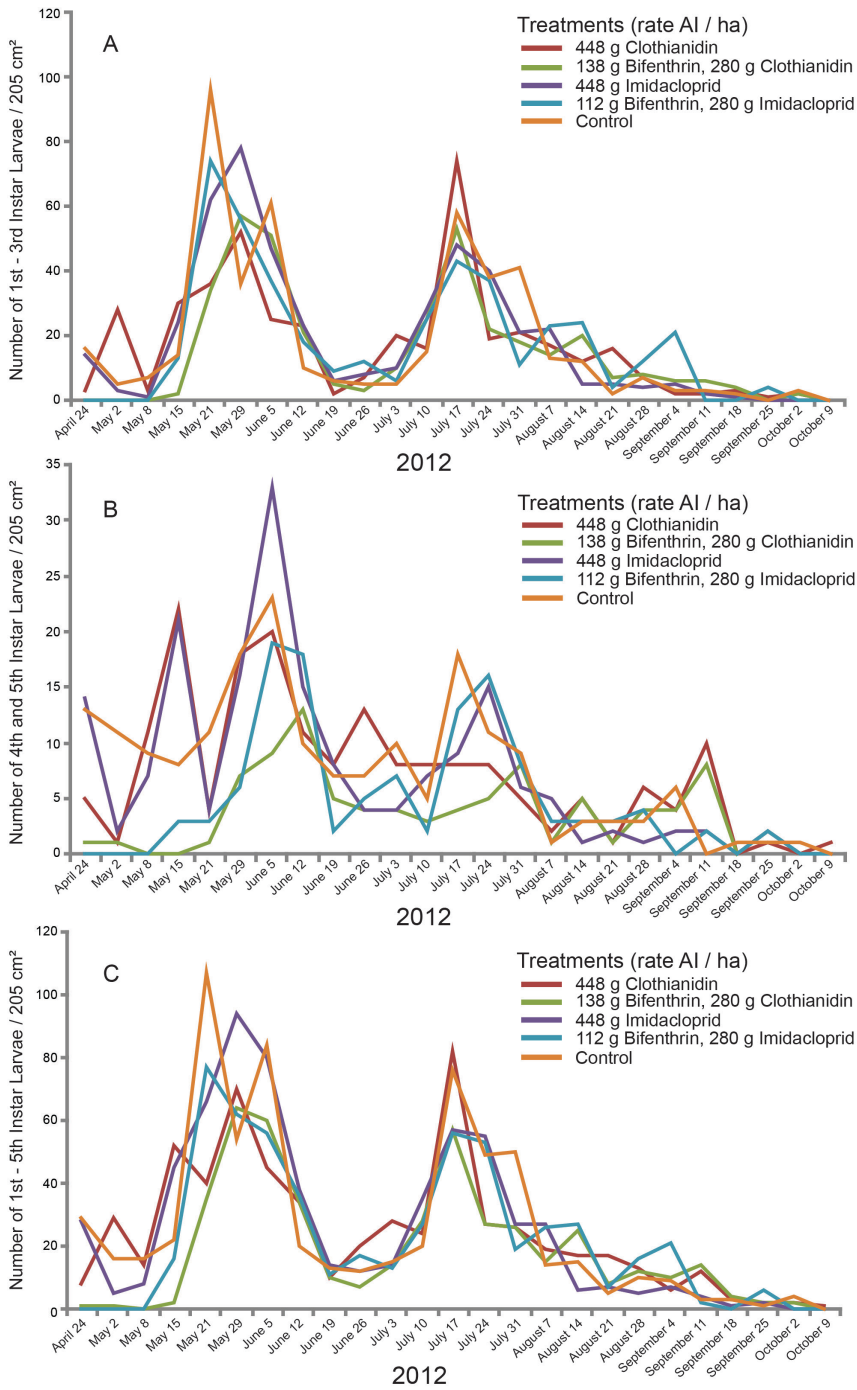


Fig. 21. Season-long number of 1st-3rd (A), 4th-5th (B), and 1-5th (C) instar larvae per 205 cm² of turfgrass in Arena (448 g clothianidin / ha), Aloft (139 g bifenthrin / 280 g clothianidin / ha), Merit (448 g imidacloprid / ha), Allectus (112 g bifenthrin / 280 g imidacloprid / ha) and control treatments from Baltic, CT, 2012.

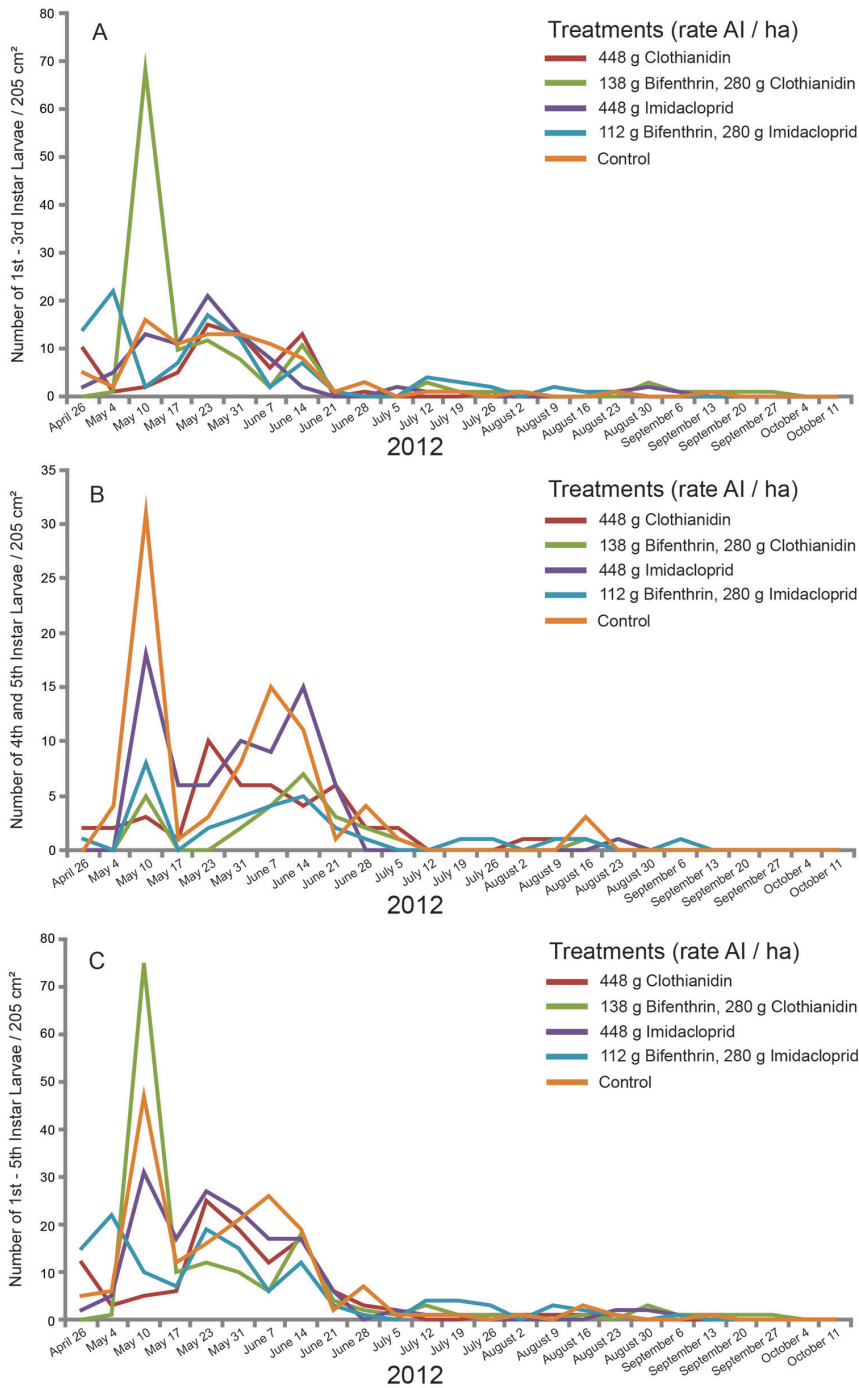


Fig. 22. Season-long number of 1st-3rd (A), 4th-5th (B) and 1-5th (C) instar larvae per 205 cm² of turfgrass in Arena (448 g clothianidin / ha), Aloft (139 g bifenthrin / 280 g clothianidin / ha), Merit (448 g imidacloprid / ha), Allectus (112 g bifenthrin / 280 g imidacloprid / ha) and control treatments from Westerly, RI, 2012.