

Clothianidin and Imidacloprid Residues in *Poa annua* (Poales: Poaceae) and Their Effects on *Listronotus maculicollis* (Coleoptera: Curculionidae)

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ABSTRACT Competitive enzyme-linked immunosorbent assay was used to quantify the amounts of the neonicotinoids clothianidin and imidacloprid in *Poa annua* L. clippings from treated golf course fairways. Average clothianidin residues 7 d after application ranged from 674 to 1,550 ng/g tissue in 2012 and 455–2,220 ng/g tissue in 2013. Average clothianidin residues the day of application ranged from 17,100–38,800 ng/g tissue in 2014. Average imidacloprid residues 7 d after treatment ranged from 1,950–3,030 ng/g tissue in 2012 and 7,780–9,230 ng/g tissue in 2013. Average imidacloprid residues the day of application ranged from 31,500–40,400 ng/g tissue in 2014. Neonicotinoid or bifenthrin–neonicotinoid combination products applied in field plots in 2012 did not significantly reduce the numbers of larvae relative to the untreated control. However, in 2013, statistically significant reductions in the numbers of larvae recovered from treated field plots were associated with the presence of bifenthrin alone or when used in combination with neonicotinoid active ingredients. *Listronotus maculicollis* (Kirby) adults caged on neonicotinoid-, bifenthrin-, and bifenthrin–neonicotinoid-treated *P. annua* turf plugs fed on *P. annua* leaves, but mortality was only highly significantly different between treated and untreated foliage when weevils were placed on treated foliage the day after treatment and allowed to feed for 7 d. The modest degree of population suppression with bifenthrin in these experiments may not be adequate to justify the continued use of these products due to the increased risk of insecticide resistance and disruption of biological control.

KEY WORDS ELISA, clothianidin, imidacloprid, bifenthrin, *Listronotus maculicollis*

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, as they feed at the plant crown. Where larval densities exceed 15 per 458 cm² (0.5 foot²), injury to golf course greens, collars, and fairways is common (McGraw and Koppenhöfer 2008). Instars 1–3 feed inside plant stems while

fourth and fifth instars feed on plant crowns. There are normally three generations of *L. maculicollis* per year in the northeastern United States.

Fourth-generation pyrethroids have 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 sought to determine the concentrations of systemic clothianidin and imidacloprid in *P. annua* tissue following field applications, and evaluate their toxicity (along with neonicotinoid–bifenthrin combination products and bifenthrin alone) to larvae and adults.

Materials and Methods

Fairway Treatments, *P. annua* Tissue and Larval Sampling 2012. Four replicates of the following treatments: 1.05 liters/ha Aloft SC (138 g bifenthrin, 280 g clothianidin; Arysta LifeScience, Cary, NC), 0.89

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kg/ha Arena 50WDG (448 g clothianidin; Valent USA Corp., Walnut Creek, CA), 5.26 liters/ha of Allectus SC (112 g bifenthrin, 280 g imidacloprid; Bayer Environmental Science, Research Triangle Park, NC), and 1.87 liters/ha Merit 2 F (448 g imidacloprid; Bayer Environmental Science) were applied in 2012 in a randomized complete block design, with 6- by 6-m plots on one fairway in Baltic, CT (17 April) and repeated on a single fairway in Westerly, RI (19 April). We used CO₂ sprayers at 280 kPa and four-nozzle wands equipped with 8003VS TeeJet nozzles (Spraying Systems Co., Wheaton, IL) and applied treatments using 408 liters/ha of water.

Larval and *P. annua* tissue sampling began one week after application of insecticide treatments and continued weekly for 25 wk. A 0.94- by 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 was attached to the frame to make 230 (5.72 by 5.72 cm) squares equal to the diameter (5.72 cm) of the turf plug extractor. Flags were randomly placed in squares to mark where turf plug samples would be taken. Records of previous samples were kept to prevent sampling again from the same location. At both sites (Baltic, CT; Westerly, RI), three turf plug samples were taken from each plot weekly. Two turf plugs from each plot were placed in modified Berlese funnels to collect larvae (Diaz et al. 2008). Glycerin (5 ml) was used in the bottom collection containers to preserve larvae. Funnel containers were checked up to 14 d after collection and larvae were removed and placed in vials of 70% ethanol until head capsule widths could be measured. Head capsule width was measured using a binocular stereo microscope fitted with an eyepiece reticle at 63× magnification to determine larval instars (Diaz et al. 2008). The third plug from each plot was used to provide grass clippings for enzyme-linked immunosorbent assay (ELISA) measurements of neonicotinoids present. 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.

Fairway Treatments, *P. annua* Tissue and Larval Sampling 2013. Eight replicates 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 (112 g bifenthrin, 280 g imidacloprid), 1.87 liters/ha Merit 2 F (448 g imidacloprid) and Talstar S 1.73 liters/ha (138 g bifenthrin; FMC Corp., Philadelphia, PA) were applied in a randomized complete block design on 14 May 2013, with 1.5- by 3-m plots on a single golf course fairway in Baltic, CT. We used CO₂ sprayers at 280 kPa and single nozzle wands equipped with 8002EVS TeeJet nozzles (Spraying Systems Co.) and applied treatments using 815 liters/ha of water. Four replicates of the same treatments were applied to a single golf course fairway in a randomized complete block design in Westerly, RI, on 20 May 2013.

P. annua tissue sampling began 1 wk after application and continued weekly for 4 wk. At least 0.5 g of

tissue was clipped from each treated plot, then placed in a labeled plastic bag and stored at -20°C until analysis. Samples for larval counts from Baltic, CT, were taken on 4 June 2013 and from Westerly, RI, on 3 June 2013. The samples consisted of taking five 10.8-cm-diameter plugs per replicate and submerging them in a saturated salt solution. Larvae that floated were collected, and instars were determined by measuring head capsule widths.

Fairway Treatments and Turf Plug Assays With Adults 2014. Four replicates of the following treatments plus a water only control were arranged in a randomized complete block design: 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 (112 g bifenthrin, 280 g imidacloprid), 1.87 liters/ha Merit 2 F (448 g imidacloprid), and Talstar S 1.73 liters/ha (138 g bifenthrin). Treatments were applied on 27 May 2014 to a single golf course fairway in Westerly, RI, using CO₂ sprayers at 280 kPa and single-nozzle wands equipped with 8002EVS TeeJet nozzles (Spraying Systems Co.) and applied using 815 liters/ha of water. *P. annua* clippings from treated plots were collected the day of treatment (0 DAT), 7 and 14 DAT and kept at -20°C. One turf plug (5.72 cm diameter) was taken from each of the four replicates 0 and 7 DAT and from three replicates 14 DAT and brought back to the laboratory and placed in 120-ml plastic cups. Ten adult weevils (from the same golf course where the treatments were applied) were placed on each plug and the cups were covered with a screened lid. Adult weevil mortality was evaluated 7 d after being placed on plugs.

ELISA Methods. Samples of finely chopped grass clippings were weighed (0.5 g per sample) and added to a 15-ml centrifuge tube containing 5 ml of 100% methanol. The samples were gently shaken overnight at room temperature. Insecticide concentrations were quantified using 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) and then reconstituted in a 0.05% aqueous solution of Triton X-100 before analysis by ELISA. The reconstituted samples were used directly for ELISA or were further diluted with 0.05% Triton X-100 to bring the concentrations of insecticide within the detection range of the ELISA kits (0.2–6 ng/ml imidacloprid and 1.5–15 ng/ml clothianidin). The final concentrations of insecticide were converted to nanogram of insecticide per gram of plant tissue (ng/g is parts per billion, or ppb). A Molecular Devices SpectraMAX 250 microplate reader (Sunnyvale, CA) was used to read plates.

Statistical Analysis. Larval data were analyzed by analysis of variance (ANOVA) followed by mean separation by Tukey's HSD test (SAS 2003). Live and dead adults from each evaluation date and treatment were combined across replicates; numbers of

Table 1. *P. annua* tissue residue concentrations for neonicotinoid insecticides and mortality in groups of 10 adult annual bluegrass weevil after feeding on these tissues for 7 d

Active Ingredient	AI/ha (g)	0 DAT		7 DAT		14 DAT	
		Concentration ^a (ng/g)	Mortality ^b (%)	Concentration (ng/g)	Mortality (%)	Concentration (ng/g)	Mortality (%)
Clothianidin	448	38800 ± 7578	28 ± 6***	1400 ± 183	18 ± 9	384 ± 58	13 ± 8
Clothianidin	280	17090 ± 2144	30 ± 1***	558 ± 92	23 ± 13	<200	23 ± 3*
Bifenthrin	138						
Imidacloprid	280	31500 ± 2544	28 ± 9***	3160 ± 787	8 ± 5	1690 ± 211	10 ± 5
Bifenthrin	112						
Imidacloprid	448	40430 ± 5586	3 ± 3	6180 ± 522	18 ± 5	2700 ± 724	13 ± 3
Bifenthrin	138	-	13 ± 5*	-	23 ± 8	-	13 ± 6
Control	-	-	0 ± 0	-	15 ± 3	-	3 ± 3

Plots were sprayed 27 May and samples were taken the same day (0 DAT, days after treatment), 3 and 10 June (7 and 14 DAT, respectively). Values given are means ± SE, n = 4 for 0 and 7 DAT, n = 3 for 14 DAT.

^a Active ingredient: imidacloprid or clothianidin depending upon the treatment applied.

^b Means in the same column followed by an asterisk(s) are significantly different from the control (Fisher's Exact Test, P ≤ 0.05 (*), 0.01 (**), and 0.001 (***)).

live and dead weevils for each treatment were compared with the untreated control group with Fisher Exact Test, using the two-tailed probability (Microsoft Research, 2014). Percent mortality data with standard errors for the replicates are reported in Table 1.

Results

Clothianidin Residues in *P. annua* Tissue. Average clothianidin residues in *P. annua* tissue 7 d after treat-

ment ranged from 1,030 (Baltic, CT) to 1,550 (Westerly, RI) ng/g where 448 g/ha of clothianidin was applied in 2012 (Fig. 1A). In 2013, residue concentrations ranged from 547 (Westerly, RI) to 2,220 (Baltic, CT) ng/g (Fig. 1C). At the lower application dosage (280 g/ha of clothianidin) the average clothianidin residues 7 d after treatment ranged from 674 (Baltic, CT) to 1,150 (Westerly, RI) ng/g in 2012 (Fig. 1B); concentrations ranged from 455 (Westerly, RI) to 1,450 (Baltic, CT) ng/g in 2013 (Fig. 1D). In all of these treated plots, the concentrations of clothianidin

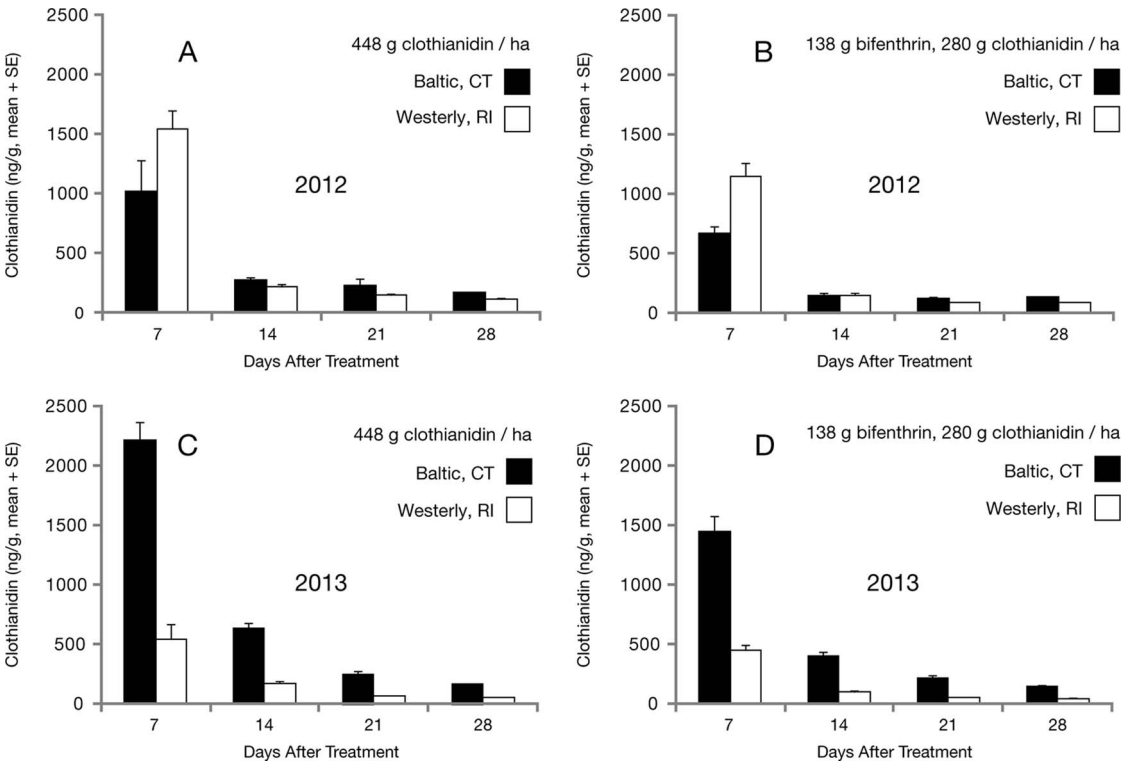


Fig. 1. Clothianidin concentration in fresh weight of *P. annua* leaf tissue (ng/g, mean + SE) where treatments containing 448 g clothianidin (A and C) or 138 g bifenthrin and 280 g clothianidin (B and D) were applied per hectare in 2012 and 2013 on golf course fairways in Baltic, CT, and Westerly, RI.

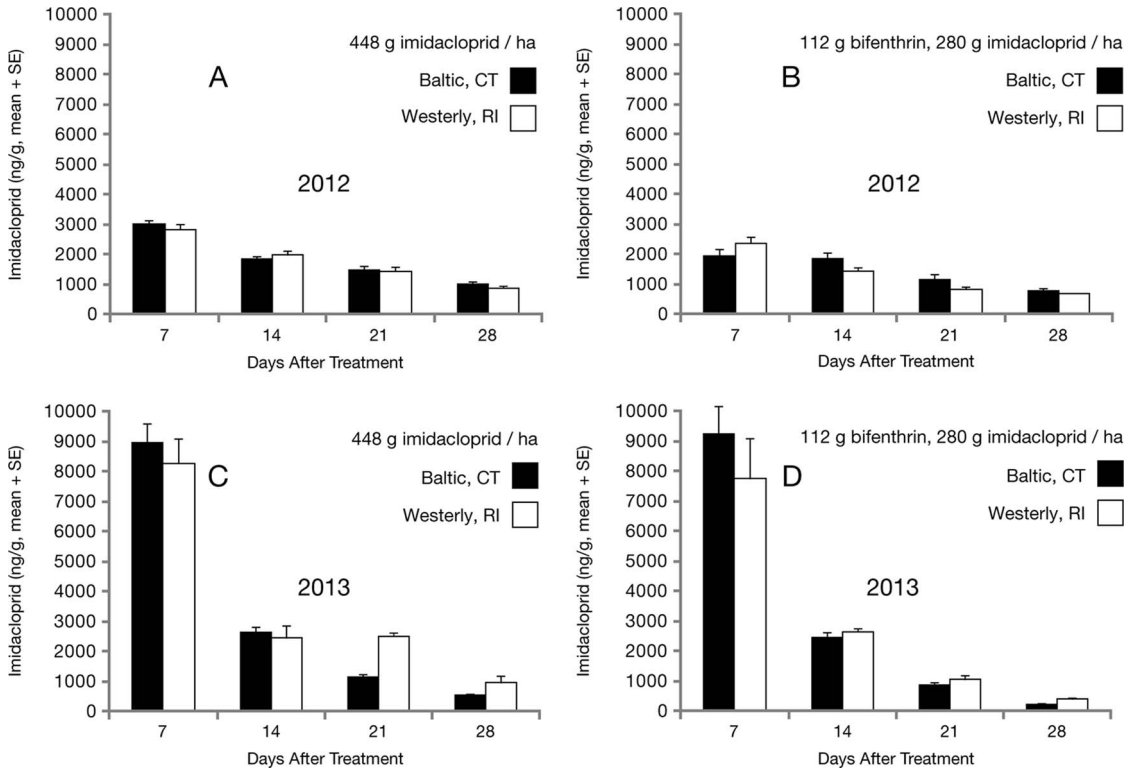


Fig. 2. Imidacloprid concentration in fresh weight of *P. annua* leaf tissue (ng/g, mean + SE) where treatments containing 448 g imidacloprid (A and C) or 112 g bifenthrin and 280 g clothianidin (B and D) were applied per hectare in 2012 and 2013 on golf course fairways in Baltic, CT, and Westerly, RI.

had dropped to levels near the lower detection limit of the ELISA (200 ppb) by 4 wk after the initial application dates (Fig. 1A–D).

Imidacloprid Residues in *P. annua* Tissue. The average imidacloprid residues in *P. annua* tissue 7 d after treatment ranged from 2,850 (Westerly, RI) to 3,030 (Baltic, CT) ng/g where 448 g/ha of imidacloprid was applied in 2012 (Fig. 2A). Residues ranged from 8,260 (Westerly, RI) to 8,970 (Baltic, CT) ng/g in 2013 (Fig. 2C). At the lower application dosage (280 g/ha of imidacloprid), imidacloprid residues in *P. annua* tissue 7 d after treatment ranged from 1,950 (Baltic, CT) to 2,350 (Westerly, RI) ng/g in 2012 (Fig. 2B). In 2013, concentrations ranged from 7,780 (Westerly, RI) to 9,230 (Baltic, CT) ng/g (Fig. 2D). By 4 wk after treatment, the levels of imidacloprid were still above the lower detection limit of the ELISA (75 ppb) but had dropped by 66% (Baltic, CT) and 70% (Westerly, RI) where 448 g/ha of imidacloprid had been applied in 2012 (Fig. 2A). At the 280 g/ha dosage, the levels of imidacloprid had dropped by 59% (Baltic, CT) and 72% Westerly, RI (Fig. 2B). In 2013, imidacloprid levels had dropped by 94% (Baltic, CT) or 88% (Westerly, RI) four weeks after treatment where 448 g/ha of imidacloprid had been applied (Fig. 2C). In 2013, imidacloprid levels had dropped by 97% (Baltic, CT) or 95% (Westerly, RI) four weeks after treatment at the 280 g/ha dosage (Fig. 2D).

Larval Suppression 2012. There were no significant differences in larval mortality between treatment and untreated control plots in 2012 at either Baltic, CT, or Westerly, RI ($F = 0.89$; $df = 4,12$; $P = 0.50$ and $F = 0.34$; $df = 4,12$; $P = 0.84$, respectively).

Larval Suppression 2013. Larval population densities in the imidacloprid and clothianidin treatments at Baltic, CT, were not significantly different from untreated control plots. There was also no significant difference in first–third instars between the clothianidin–bifenthrin treatment and untreated control plots. However, all other treatments containing bifenthrin had significantly fewer first–third ($F = 9.80$; $df = 5,35$; $P < 0.01$), fourth and fifth ($F = 9.63$; $df = 5,35$; $P < 0.01$), and first–fifth instars ($F = 11.21$; $df = 5,35$; $P < 0.01$) than untreated control plots (Fig. 3). Larval population densities of first–fifth instars in the imidacloprid, clothianidin, and bifenthrin alone treatments at Westerly, RI, did not differ significantly from untreated control plots (Fig. 4). Neither were there any significant differences in fourth–fifth instars between treated and untreated control plots. However, the imidacloprid–bifenthrin treatment had significantly fewer first–third instars than the untreated control plots (Fig. 4). The imidacloprid–bifenthrin and clothianidin–bifenthrin treatments did have significantly fewer first–fifth instars ($F = 3.51$; $df = 5,15$; $P = 0.03$) than untreated control plots (Fig. 4).

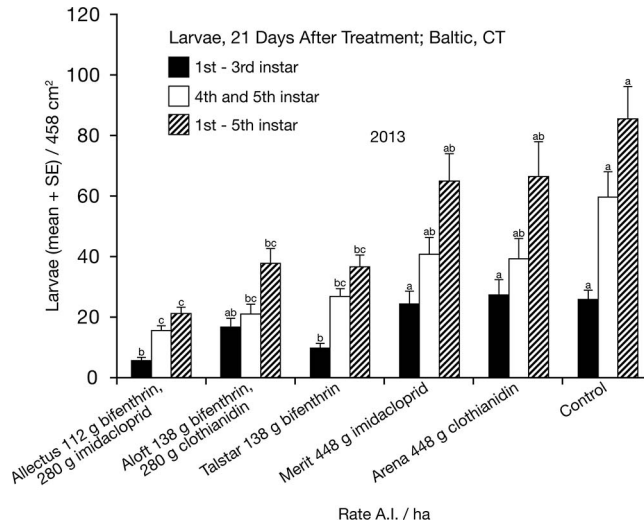


Fig. 3. Number of first-third, fourth and fifth, and combined first-fifth instars (mean + SE) where 112 g bifenthrin + 280 g imidacloprid, 138 g bifenthrin + 280 g clothianidin, 138 g bifenthrin, 448 g imidacloprid, or 448 g clothianidin were applied per hectare in Baltic, CT, 2013. Means were compared among instar groupings (first-third, fourth-fifth, first-fifth; $P = 0.05$, Tukey's HSD test; $n = 8$).

Adult Mortality. There was significant mortality of *L. maculicollis* adults when they were placed on clothianidin-, clothianidin-bifenthrin-, and imidacloprid-bifenthrin- (average neonicotinoid concentrations of 38,800; 17,100; and 31,500 ng/g, respectively) treated foliage the day after treatment and allowed to feed for 7 d (Table 1). When adults were placed on treated foliage 7 d after treatment and held for 7 d, there was no significant mortality (Table 1). When adults were placed on treated foliage 14 d after treatment and held for 7 d, there was significant mortality between the

clothianidin plus bifenthrin combination treatment and the control (Table 1).

Discussion

The long soil half-lives of clothianidin and imidacloprid, 545 and 191 d respectively (University of Hertfordshire 2013), led us to test the hypothesis that if these materials were applied early in the season to control overwintering adults, the amounts inside *P. annua* tissue would still be high enough later in the

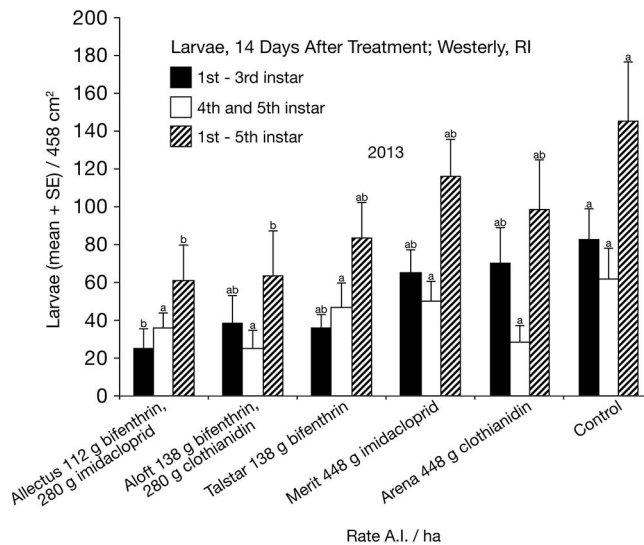


Fig. 4. Number of first-third, fourth and fifth, and first-fifth instars (mean + SE) where 112 g bifenthrin + 280 g imidacloprid, 138 g bifenthrin + 280 g clothianidin, 138 g bifenthrin, 448 g imidacloprid, or 448 g clothianidin were applied per hectare in Westerly, RI, 2013. Means were compared among instar groupings (first-third, fourth-fifth, first-fifth; $P = 0.05$, Tukey's HSD test; $n = 4$).

season to control first-generation larvae. This does not appear to be the case. Our data show $\approx 30\%$ mortality of adults from the highest field rate of clothianidin and the combination products (clothianidin–bifenthrin and imidacloprid–bifenthrin) within a week of treatment (Table 1). This was probably due to the high rate of clothianidin where that treatment was used alone and the bifenthrin in the combination products. This level of mortality does not support the strategy that superintendents should adopt very early-season neonicotinoid treatments of *P. annua* plants to control adult weevils which are emerging from overwintering sites as they begin to feed. The lack of adult mortality from bifenthrin alone and the combination products that contained bifenthrin 1 wk after treatment suggests that the weevils may have been resistant to bifenthrin.

First through third instars feed inside *P. annua* stems while fourth and fifth instars feed on plant crowns. Koppenhöfer et al. (2012) found that applications of clothianidin or imidacloprid between 15 April and 3 May provided an average of 54 and 48% control, respectively, whereas applications between 18 May and 10 June provided averages of 64 and 78% control, respectively. In our studies, it appears that either we applied these products earlier than optimal to effect significant control in 2012, or that the populations of annual bluegrass weevil we studied were relatively insensitive to neonicotinoids. The later applications in 2013 (27 d later at Baltic, CT, and 31 d later at Westerly, RI) did result in greater larval mortality, suggesting that application timing was the more likely cause of the poor control in 2012. The higher mortality in 2013 (although still not adequate to reduce populations below the damage threshold) is likely due to the greater amounts of neonicotinoids present when a greater portion of the first-generation instars 1–3 were present. Adult mortality arising from the bifenthrin treatments could also have contributed to the reduction in larval numbers.

Other studies (Koppenhöfer et al. 2012) have shown levels of 54–64% field population reductions from clothianidin and 48–78% control from imidacloprid alone depending upon rate and application timing. It is unknown whether reported significant treatment effects in the field, measured as reduced larval population, is due to contact with adults, behavioral changes, reduced fecundity, or larval mortality. In petri dish assays, we found that clothianidin, imidacloprid, and dinotefuran (another neonicotinoid) had a very fast knockdown effect but adults recovered within 24 h (C. C., unpublished data). Reduced response to neonicotinoids (multiple resistance) in the populations we studied is a possibility, compared with those where significant treatment effects have been reported (Koppenhöfer et al. 2012).

The greatest imidacloprid residues present in *P. annua* 7 d after treatment in 2013 (8,970 ng/g tissue at Baltic, CT, and 8,260 ng/g at Westerly, RI) were found following application of 448 g/ha imidacloprid and 9,230 and 7,780 ng/g where 280 g/ha imi-

daclorid was applied with 112 g/ha bifenthrin at Baltic, CT, and Westerly, RI, respectively. The formulation of imidacloprid alone was a flowable while the combination product was a soluble concentrate. Similarly, some of the greatest clothianidin residues present in *P. annua* tissue 7 d after treatment in 2013 (2,220 ng/g tissue at Baltic, CT, and 547 ng/g at Westerly, RI) were found where 448 g/ha clothianidin had been applied alone. When 280 g/ha clothianidin was applied with 138 g/ha bifenthrin, 1,450 and 455 ng/g were found in *P. annua* tissue 7 d after treatment at Baltic, CT, and Westerly, RI, respectively. The formulation of clothianidin alone was a water dispersible granule while the combination product was a soluble concentrate. Because the lower application rates (280 g) of the soluble concentration formulated products approached or even exceeded (9,230 ng/g imidacloprid) the levels found in *P. annua* tissue from flowable or water dispersible granule formulations (448 g application dosage per ha), it appears that the soluble concentrate formulations allow greater mobility of neonicotinoids into plant tissue. It is important to note, however, that the ELISA method used does not distinguish between internal and external residues of the neonicotinoids. Thus, the higher concentrations measured immediately after the treatments were applied (in 2014) are more likely to reflect surface residues. Mowing frequency and irrigation may have a significant impact on these residues, as removal of mowed grass and irrigation could deplete available residues before they are absorbed by the grass.

We made the insecticide applications at Baltic, CT, on 17 April and to Westerly, RI, on 19 April in 2012. In 2013, we made the insecticide applications to Baltic, CT, on 14 May and to Westerly, RI, on 20 May. Applications were made approximately 1 mo later in 2013 than in 2012. The difference between 1,000 ppb clothianidin 7 d after treatment at Baltic in 2012 and 2,100 ppb in 2013 may be explained by a 8.1-cm rainfall 6 d after treatment at that site in 2012. The water solubility of clothianidin is fairly high (340 ppm) and could have washed off plant surfaces (Pesticide Properties Database, 2014).

The difference between 1,500 ppb clothianidin 7 d after treatment at Westerly, RI, in 2012 and 500 ppb in 2013 can be explained by the increased frequency of mowing at the later date in 2013. The courses at Baltic, CT, and Westerly, RI, are quite different in their microclimates. One is an inland course (Baltic, CT) and warms up much quicker in the spring than the seaside course in Westerly, RI. The degree-day accumulation differences between these two sites translates into differences in plant growth which further translates into differences in mowing frequency. The differing amounts of clothianidin from year to year and site to site shows the difficulty in predicting levels of control to be expected with these applications in any given year.

The level of clothianidin 7 d after treatment where 138 g/ha bifenthrin and 280 g/ha clothianidin had

been applied in Baltic, CT, in 2013 was 1,450 ng/g while the level in the 448 g/ha clothianidin treatment was 2,220 ng/g 7 d after treatment. The level of clothianidin 7 d after treatment where 138 g/ha bifenthrin and 280 g/ha clothianidin had been applied in Westerly, RI, in 2013 was 455 ng/g while the level in the 448 g/ha clothianidin treatment was 547 ng/g 7 d after treatment. At both sites the lower application levels of clothianidin when combined with bifenthrin showed significant reductions in larval populations from the untreated control. This effect is most likely due to the bifenthrin because higher application dosages of clothianidin applied alone did not result in significant reductions in larval numbers.

The average imidacloprid residue 7 d after treatment where 112 g/ha bifenthrin and 280 g/ha imidacloprid had been applied in Baltic, CT, in 2013 was 9,230 ng/g while the average amount in the 448 g/ha imidacloprid treatment was 8,970 ng/g 7 d after treatment, a relatively small difference of 260 ng/g. The mean amount of imidacloprid 7 d after treatment where 112 g/ha bifenthrin and 280 g/ha imidacloprid had been applied in Westerly, RI, in 2013 was 7,780 ng/g while the mean amount in the 448 g/ha imidacloprid treatment was 8,260 ng/g 7 d after treatment. At both sites the lower application levels of imidacloprid when combined with bifenthrin showed significantly greater larval reduction from the control, while larval reduction where the higher application levels of imidacloprid alone had been applied were not significantly different from the control. As with clothianidin, it appears that the addition of bifenthrin in the combination product with imidacloprid is primarily responsible for the modest reduction in larval numbers.

Koppenhöfer et al. (2012) reviewed 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/ha. The majority of applications were with either 337 g/ha (32 trials) or 448 g/ha (14 trials). Four of the 337 g/ha applications showed zero percent control, even though they were applied when some level of control of larvae would be expected. Similarly, 49 Arena applications (46 were 50 WDG and 3 were 0.5G formulations) with clothianidin rates between 168 and 449 g/ha were analyzed. The majority of these applications were either 224 g/ha (13 trials) or 449 g/ha (15 trials). Two of the 280 g/ha, one 337 g/ha, and one 449 g/ha applications showed zero percent control when some level of control would be expected. Koppenhöfer et al. (2012) found that several populations could be labeled as resistant to pyrethroids, organophosphates, neonicotinoids, indoxacarb, and bifenthrin–neonicotinoid combination products. If the populations at Baltic, CT, and Westerly, RI, were among those that demonstrated multiple resistance, this could explain the lack of significant weevil mortality in our 2012 experiments.

We judge that it is unwise for superintendents to continue applying pyrethroid products, including

combination products with neonicotinoids, where there is evidence of pyrethroid resistance. Such applications will continue intensive selection for metabolic detoxification mechanisms, which Ramoutar et al. (2009b) demonstrated to involve increasing enzymatic complexity, as the LD₅₀ for pyrethroids increases. Involvement of cytochrome P450 monooxygenase enzymes, carboxylesterases, and glutathione transferase families of enzymes may heighten the risk of multiple resistance across insecticide mode of action classes. Furthermore, combinations of broad-spectrum insecticides may have the unintended ecological consequence of suppressing natural enemies (e.g., ants) of annual bluegrass weevils. These predators may provide the only biological “safety net” for preventing turf destruction from insecticide-resistant weevils.

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References Cited

- Britton, W. E. 1932. A new pest of lawns. *Conn. Agric. Exp. Sta. Bull.* 52: 25–28.
- Diaz, M., M. Seto, and D. C. Peck. 2008. Patterns of variation in the seasonal dynamics of *Listronotus maculicollis* (Coleoptera: Curculionidae) populations on golf course turf. *Environ. Entomol.* 37: 1438–1450.
- Koppenhöfer, A. M., S. R. Alm, R. S. Cowles, B. A. McGraw, S. Swier, and P. J. Vittum. 2012. Controlling annual bluegrass weevil: optimal insecticide timing and rates. *GCSAA Golf Course Manag. Mag.* 80: 98–104.
- McGraw, B. A., and A. M. Koppenhöfer. 2008. Evaluation of two endemic and five commercial entomopathogenic nematode species (Rhabditida: Heterorhabditidae and Steinernematidae) against annual bluegrass weevil (Coleoptera: Curculionidae) larvae and adults. *Biol. Control* 46: 467–475.
- Microsoft Research. 2014. Fisher's exact test calculator for 2 × 2 contingency tables. (<http://research.microsoft.com/en-us/um/redmond/projects/mscompbio/fisherexacttest/>).
- Ramoutar, D., S. R. Alm, and R. S. Cowles. 2009a. Pyrethroid resistance in populations of *Listronotus maculicollis* (Coleoptera: Curculionidae) from southern New England golf courses. *J. Econ. Entomol.* 102: 388–392.
- Ramoutar, D., R. S. Cowles, and S. R. Alm. 2009b. Pyrethroid resistance mediated by enzyme detoxification in *Listronotus maculicollis* (Coleoptera: Curculionidae) from Connecticut. *J. Econ. Entomol.* 102: 1203–1208.
- Ramoutar, D., R. S. Cowles, E. Requentina, Jr., and S. R. Alm. 2010. Synergism between demethylation inhibitor fungicides, or gibberellin inhibitor plant growth regulators and bifenthrin in a pyrethroid-resistant population of

- Listronotus maculicollis* (Coleoptera: Curculionidae). *J. Econ. Entomol.* 103: 1810–1814.
- SAS Institute.** 2003. SAS, version 9.2. SAS Institute, Cary, NC.
- Tashiro, H.** 1976. A serious menace to *P. annua* in the Northeast. *Golf Supt. March*: 44(3): 34–37.
- University of Hertfordshire.** 2013. The pesticide properties database (PPDB) developed by the Agriculture & Environment Research Unit (AERU), University of Hertfordshire, 2006–2013. University of Hertfordshire, Hatfield, United Kingdom.
- Vittum, P. J.** 2005. Annual bluegrass (*Hyperodes*) weevil – resistance to pyrethroids or not? *Univ. Mass. Ext. Turf Prog. Manag. Update.* Aug. 24. (http://www.umasssturf.org/management_updates/2005_archive/05aug24.htm).
- Vittum, P. J., M. G. Villani and H. Tashiro.** 1999. *Turfgrass insects of the United States and Canada.* 2nd ed. Cornell University Press, Ithaca, New York, NY.

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