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Effects of clothianidin-treated seed on the arthropod community in a mid-Atlantic no-till corn agroecosystem

Heather H Disque,[a] Kelly A Hamby,[b] Aditi Dubey,[b] Christopher Taylor[b] and Galen P Dively[b*]

Abstract

BACKGROUND: Nearly all corn seed in the US is coated with neonicotinoid insecticides to protect against soil and foliar arthropod pests. Exposure in the soil and the systemic activity in the plant can pose non-target risks. We assessed the community-level effects of clothianidin-treated seed on the diversity and abundance of arthropod communities in a no-till corn agroecosystem over a single growing season.

RESULTS: Epigeal and foliage-dwelling communities were disturbed by the clothianidin seed treatment, with significant negative and positive changes in taxa abundances. Clothianidin reduced the abundance of minute pirate bugs by 66.2%, lady beetles by 44.7%, ants by 43.4%, ground beetle adults and larvae by 31.7%, and rove beetles by 44.1% during the early corn growth stages. Herbivores, particularly thrips, were more negatively affected by clothianidin than other trophic groups. In contrast, some groups, such as collembolans and leafhoppers, exhibited significantly higher abundances in the seed treated plots.

CONCLUSION: Clothianidin primarily influenced arthropod communities during the 4 weeks following planting, with disruptions to major natural enemy taxa, but communities showed trends toward recovery at the later corn stages. While the insecticide suppressed multiple herbivores, none were economically damaging to corn; thus, the pest suppression benefits of clothianidin observed in this study did not justify the non-target impacts.
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Supporting information may be found in the online version of this article.

Keywords: clothianidin; seed treatment; non-target effects; community responses; no till corn system

1 INTRODUCTION

Currently, more than 90% of all corn seed in the US is coated with neonicotinoid insecticides to protect against soil and foliar arthropod pests.[1–3] Widespread use of these reduced risk insecticides[4] occurs due to their prophylactic nature, convenience, and low cost.[5] The relatively low dose applied to seed is assumed to be less disruptive to non-target organisms than foliar-applied insecticides.[6] However, their presence on the seed coating as well as the systemic activity in the plant can pose non-target risks.[7]

Negative effects from direct and indirect exposure to neonicotinoids have been reported for a number of beneficial insects including: Coleoptera: Coccinellidae, Carabidae, Staphylinidae[8–12]; Hemiptera: Anthocoridae, Lygaeidae, Pentatomidae, Reduviidae[13–15]; Neuroptera: Chrysopidae[14,16] and hymenopteran parasitoids.[13,17–20] Furthermore, a meta-analysis of 20 field studies[21] reported an overall approximately 16% reduction in natural enemy abundance, which was comparable to the impact caused by foliar and soil-applied pyrethroids. Studies have also demonstrated non-target effects to bees (Hymenoptera: Apoidea) *via* exposure to contaminated dust during planting, guttation droplets exuded from treated corn seedlings, and residues in pollen and nectar.[22–34] With only 1.6–20% of the active ingredient taken up by the plant,[7,35] the persistence and environmental fate of neonicotinoid parent compounds and metabolites pose potential risks to soil invertebrates and microbial communities.[36–40] Furthermore, detectable residues are increasingly present in surface waters due to their high water solubility and low soil binding,[41,42] which can have negative impacts on aquatic systems.[43]

In addition to direct effects, neonicotinoid-treated seeds can indirectly affect beneficial arthropods by reducing their prey populations. Many generalist parasitoids and predators respond to herbivores that colonize crop plants early in the growing season before pest species are present.[44,45] In the mid-Atlantic US, thrips (Thysanoptera) and other non-pest herbivores colonize seedling and whorl stages of corn but rarely cause economic damage. Early

* *Correspondence to: GP Dively, 4112 Plant Sciences Bldg., Department of Entomology, University of Maryland, College Park, MD 20742, USA. E-mail: galen@umd.edu*

a *Maryland Department of Agriculture, Easton, MD, USA*

b *Department of Entomology, University of Maryland, College Park, MD, USA*

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season suppression of these herbivores by the systemic activity of neonicotinoids may delay colonization and establishment of beneficial arthropods, resulting in higher pest populations later in the season. For example, presence of alternative thrips prey promoted *Orius insidiosus* (Say) (Hempitera: Anthocoridae) colonization of soybeans prior to the arrival of soybean aphid *Aphis glycines* (Matsumura) in Indiana, and outbreaks only occurred when soybean aphids arrived before establishment of *O. insidiosus*.[45] Other studies have shown that neonicotinoid treatments can trigger outbreaks of the twospotted spider mite *Tetranychus urticae* (Koch) by increasing its fecundity, reducing natural enemy pressure, and modifying leaf physiology in favor of these pests.[46,47]

The majority of studies on non-target effects of neonicotinoid seed treatments have focused on imidacloprid, the oldest and most widely used active ingredient; however, thiamethoxam and clothianidin, of which the latter is the principal metabolite of thiamethoxam, are currently the major compounds used for seed treatments.[1,3] Additionally, non-target effects of neonicotinoid seed treatments have been largely reported on individual taxa, particularly bees and specific natural enemies, with less focus on the arthropod community as a whole. Here, we assessed the community-level effects of clothianidin treated seed on the diversity and abundance of non-target arthropods in a no-till corn agroecosystem. We used a multivariate statistical approach to test for overall community responses, especially focusing on the epigeal taxa that inhabit the surface litter in no-till systems, where exposure to clothianidin is likely to be greatest. We also determined if early season suppression of herbivores by the seed treatment delayed colonization and establishment of beneficial organisms, resulting in higher arthropod populations later in the season.

2 MATERIALS AND METHODS

2.1 Plot design and treatments

Replicated field plot experiments were conducted at each of the following two locations in 2004: Wye Research and Education Center, Queenstown, MD (38.54° N, 76.08° W), and Central Maryland Research and Education Center, Beltsville, MD (39.00° N, 76.49° W). At each site, dryland corn plots (hybrid Dekalb DKC 60-19) were planted with clothianidin-treated and untreated seed, compared side-by-side in each of four replicate blocks (total of eight plots). Plots were systematically arranged with equal numbers of each treatment in both directions of the design. The insecticide treated plots received 0.25 mg a.i. per kernel of clothianidin (Poncho 250, Bayer CropScience), which is the lowest of three doses recommended by the manufacturer and was widely used in Maryland at the time of the study. All seed was treated with a standard fungicide combination of prothioconazole (1.47%), tebuconazole (0.29%), and metalaxyl (0.59%) applied at 148 mL per 45.4 kg of seed. Plots were planted at a seeding rate to achieve 65 000 plants per ha. At Queenstown, plots measuring 27 m (36 rows) by 27 m were arranged in a linear pattern and separated by 9 m turn rows of untreated corn. At Beltsville, plots measuring 30 m (40 rows) by 36 m were arranged in strips of corn alternating with strips of wheat followed by double-cropped soybean.

2.2 Agronomic practices

Plots were planted in 0.76 m wide rows using no-tillage practices at Queenstown on 29 April and at Beltsville on 14 May. All plots were managed under a fertility program consisting of 16-8-8 starter fertilizer applied at 228 kg per ha at planting, followed by a side-dress application of 148 kg per ha of 30% nitrogen solution dribbled between the rows 3 weeks later. Paraquat (Gramoxome Extra, Syngenta Crop Protection) at 1.8 L per ha was used as a burn down herbicide, and atrazine plus S-metolachlor (Bicep II Magnum, Syngenta Crop Protection) at 4.8 L per ha was applied after planting to control weeds. The Queenstown site was adjoined by other crop fields on all sides, while the Beltsville site was surrounded by woodlots on two sides. Both sites received no other insecticide during this study and were planted with untreated soybean seed the year prior to the study.

2.3 Epigeal community

The diversity and abundance of surface-dwelling arthropods were measured using pitfall traps and litter extractions. Pitfall traps estimated population activity density over 7-day intervals at plant emergence (VE), four leaf stage (V4), and 14–21 days after anthesis (R2). At each time, two traps were placed adjacent to the furrow at an equal distance apart within each of two rows in the central sampling area (eight rows) of each plot, for a total of 32 pitfall samples per sampling date per site. Rows with traps were separated by the two center rows. Each trap consisted of two stacked 360-mL clear plastic cups buried so that the opening was level with the soil surface. The outer cup remained in place to prevent reburial when servicing the trap. The inner cup contained approximately 60 mL of undiluted ethylene glycol and was sheltered from weather and wildlife interference with a 30-cm square black plastic cover supported by three carriage bolts. After each sampling period, cups were brought back to the laboratory, and the contents were vacuum-filtered over fine organdy to remove the ethylene glycol. Captured arthropods were stored in 70% ethanol with a few drops of red food coloring and acetic acid to stain the smaller soft-bodied organisms.

Epigeal arthropods were extracted from the surface litter to provide a more quantitative assessment of the springtails, mites, dipteran larvae, and coleopteran larvae. Samples were collected at the VE and V4 stages, omitting R1 to focus on the period when insecticide exposure from the seed treatment would be the greatest. Four samples of surface litter were removed from randomly selected sites around the base of corn plants in the center of each plot. Each sample consisted of 0.2 m^2 of surface litter and the top 0.5 cm of soil. A total of 32 litter samples were taken per sampling date per site. Each sample was processed for 48 h through Berlese funnels to remove arthropods, which were collected in containers with 70% ethanol. Contents of trap and litter samples were filtered over filter paper and viewed under a stereomicroscope to identify and record arthropods to the order or family levels. Carabid beetles were further sorted and their densities recorded to the genus level; however, adult and larval stages were each combined for analysis, due to the difficulty in identifying larvae to genus, and insufficient numbers of adults for analysis at the genus level.

2.4 Foliage-dwelling community

Visual plant inspections were made at the V4, six leaf (V6), nine leaf (V9), and R2 corn growth stages to record data on the foliage-dwelling community. At each time, 25 plants were selected from the central sampling area of each plot by randomly selecting the first plant within the first row of the sampling area, and then subsequently every 10th plant along each row. Both sides of each leaf, leaf axil, tassel and silk tissue (if present) were carefully examined on each plant, starting at the base of the plant and working

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upward, recording all arthropods to the family or order levels. The stalk and developing ear were also dissected at R2 to reveal internal insects.

2.5 Aerial community

Yellow sticky cards (7.5 cm × 12.5 cm, Olson Products, Inc) were deployed for 7-day periods to estimate relative numbers of insects and other arthropods that were active in the lower corn canopy. Four cards were placed equal distances apart within the central sampling area of the plots at the VE, V4, and R2 sampling times. Cards with one sticky side exposed were attached to cane poles 20 cm above the soil surface between plants. This method was particularly efficient in assessing the diversity and abundance of flies and parasitic hymenopterans active near the ground surface. After exposure, cards were placed in a clear plastic Ziploc bag and brought to the laboratory where the captured organisms were identified under a stereomicroscope and recorded to the order or family level.

2.6 Statistical analyses

Each arthropod recorded was assigned to one of four trophic groups (saprovore, herbivore, predator or parasitoid) based on the feeding behavior of most members of its taxonomic group.[48,49] Families that did not fit within any of the trophic groups were included in the saprovore guild for analysis [*e.g.*, biting midges (Ceratopogonidae)]. For each sampling method, we characterized the overall composition of arthropod communities based on the abundance of each taxonomic group relative to the total number of arthropods recorded. The total number of taxonomic families or orders (taxa richness), number of individuals recorded (total abundance) of all taxa, and Simpson index were calculated to quantify the diversity of communities in the treated and control plots for each sampling time and method.

Principle response curve (PRC, CANOCO 5, Microcomputer Power) analysis was used to summarize all data for each sampling method to evaluate seed treatment effects on the arthropod communities as a whole.[50,51] Subsample data were averaged for each plot and both experiment sites were included in the same analysis. This was possible because the experimental design and sampling methods were identical at both locations, and the sampling times were synchronized according to corn phenology. For each community data set, the seed treatment and sampling time were set as explanatory variables, and the raw data were log (x + 1) transformed to stabilize variance and reduce the influence of dominant taxa on the ordination. Canonical coefficients were generated for each sampling time and plotted over time to show the overall response of the arthropod community to the seed treatment relative to the untreated control. Taxon-specific weights (or contributions to the observed responses) were computed to identify arthropod groups that were most affected by the seed treatment. A Monte-Carlo permutation procedure tested the null hypothesis that the canonical coefficients of the treatment response equaled zero for all sampling times. This was done by generating 499 new sets of data that were equally likely under the null hypothesis, while keeping the treatment and sampling time variables fixed. Sampling time and interactions of block and site were designated as covariables to restrict the data shuffling in a specific way to remove repeated measures and blocking effects as sources of error from the residual. The significance level was determined by the proportion of F values greater than or equal to the original F value.

Means and standard errors were calculated for the diversity measurements and the abundance of individual or pooled taxa that contributed significantly to the overall community response. Treatment effects were further analyzed by mixed model ANOVA,[52] with seed treatment and sampling time modeled as fixed factors, and block (nested within site) and site treated as random effects. The repeated measures option was used to adjust for temporally correlated observations. Before analysis, residual plots and the Shapiro-Wilk's W test were performed to examine for data normality and homogeneity of variances. Appropriate transformations or variance groupings were applied if necessary to satisfy the assumptions of ANOVA.

3 RESULTS

3.1 Epigeal community

Pitfall traps measured activity density of epigeal arthropods over a one-week period, while litter extractions provided a snapshot of total arthropod abundance. Both sampling techniques captured similar epigeal arthropod communities (Table S1 in the Supporting Information), although litter extractions showed a higher occurrence frequency of saprovores. Pooled over sampling methods and sites for fungicide-only control plots, 50 arthropod taxa were identified, of which 82.3% and 16.1% of the total were saprovores and predators, respectively. The most abundant saprovores were springtails (Isotomidae - 36.6%; Entomobryidae - 15.6%; Sminthuridae - 5.3%) and soil mites (Acari - 21.8%). In order of abundance, the predator groups of spiders (Lycosidae and Araneae), adult and larval rove beetles (Staphylinidae), adult and larval ground beetles (Carabidae), ants (Formicidae), predatory mites (Mesostigmata) and centipedes (Chilopoda) comprised 16.1% of the total combined. For pitfall samples, the mean number of taxa ($F(_{1,6}) = 9.22, P = 0.023$) and total abundance ($F_{(1,2.99)} = 31.24, P = 0.011$) were significantly higher in the clothianidin seed treated plots, while the diversity index was lower but not statistically different (Table 1). All abundance and diversity metrics for litter extracted samples did not differ by treatment.

Multivariate analyses of pitfall and litter extraction data revealed similar responses to the seed treatment over time in the epigeal communities. Only taxa that were recorded in more than 20% of the samples were included in these analyses. The first ordination axis explained 69.6% of the variance in the pitfall trap data and revealed significant negative departures in the treated community responses from the control community ($P = 0.006$). Departures indicating an overall decrease in community abundance were greatest at plant emergence, followed by increasing recovery in community structure at the V4 and R2 growth stages (Fig. 1). Similar negative departures in the treated litter community are shown by the first axis, which explained 48.9% of the variance (Fig. S1 in the Supporting Information); however, the overall effect of insecticide treated seed on arthropod abundances relative to the control community was not statistically significant ($P = 0.574$).

The contributing taxon weights given in Figures 1 and S1 in the Supporting Information indicate that several arthropod taxa were consistently related to departures from the control community. Arthropods with high positive weights (generally >0.5) followed the overall community response curve, indicating a reduction in abundance in the treated plots that decreased over time; whereas, arthropods with high negative weights (generally <−0.5) had the opposite response. In pitfall traps, overall abundances of the majority of arthropods significantly increased over the three sampling periods but were numerically lower in the insecticide

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Table 1. Summary of abundance and diversity measurements of arthropod communities recorded by sampling method in clothianidin seed treated and untreated plots of corn

Sampling method	Treatment	Mean (±SEM) per sample		
		Number of taxa	Total abundance	Simpson index
Pitfall trap	Clothianidin treated	*12.9 ± 0.41	*193.0 ± 34.9	0.5454 ± 0.02
	Untreated	*12.1 ± 0.36	*164.8 ± 21.5	0.5884 ± 0.02
Litter extractions	Clothianidin treated	10.1 ± 0.38	202.8 ± 34.7	0.4520 ± 0.03
	Untreated	10.6 ± 0.33	202.6 ± 44.1	0.4616 ± 0.02
Visual plant inspection	Clothianidin treated	8.8 ± 0.88	*128.9 ± 15.0	*0.5322 ± 0.04
	Untreated	9.7 ± 0.78	*385.3 ± 77.1	*0.3973 ± 0.05
Sticky card	Clothianidin treated	29.3 ± 0.59	150.3 ± 19.8	0.6942 ± 0.03
	Untreated	30.8 ± 0.69	177.4 ± 20.6	0.6820 ± 0.03

[a] Pairs of treated and untreated means marked with an asterisk are significantly different ($P < 0.05$).
[b] Simpson index ranges from 0 to 1, the higher value indicates greater taxa diversity.

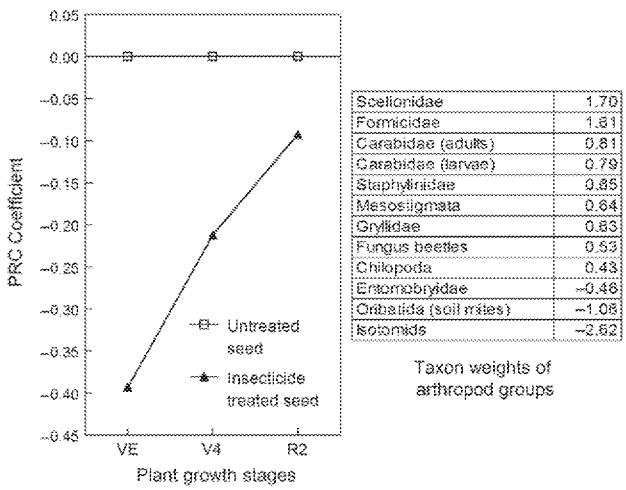

Figure 1. Principal response curve showing the effects of clothianidin insecticide-treated corn seed for the surface-dwelling arthropod community during plant emergence (VE), seedling stage (V4) and anthesis (R2). Analysis was performed on pitfall capture data as plot averages pooled over both study sites. The first ordination axis explained 69.6% of the variance in the pitfall trap data and indicated that the overall community response in the insecticide-treated plots significantly deviated from the zero reference line representing the control community ($P = 0.006$). Taxon weights indicate which arthropod groups contributed the most to the community response. Higher positive weights indicate that arthropod abundances in the insecticide-treated plots followed the trend depicted by the response curve, whereas higher negative values reveal the opposite. Most affected arthropod groups include parasitic wasps (Scelionidae), ants (Formicidae), ground beetles (Carabidae), rove beetles (Staphylinidae), predatory mites (Mesostigmata), crickets (Gryllidae), fungivore beetles (mainly Anthicidae, Nitidulidae, Mycetophagidae, and Latridiidae), centipedes (Chilopoda), springtails (Entomobryidae, Isotomidae), and soil mites (Acari). Five taxa (millipedes, globular springtails, spiders, click beetles, misc. flies) with weights near zero are not listed because their affinity to the response curve was unrelated or too weak to interpret.

treated plots, except for soil mites and springtails (Isotomidae). ANOVA results indicated significant reductions in abundance for ants (Formicidae) ($F_{(1,34.9)} = 4.57$, $P = 0.040$) by 43.4%, and for ground beetle adults and larvae (Carabidae) ($F(_{1,35}) = 5.98$, $P = 0.020$) by 31.7%. There was a significant interaction effect with sampling period on rove beetle (Staphylinidae) abundance ($F(_{2,35}) = 6.29$, $P = 0.005$), showing 44.1% and 27.2% fewer beetles in the treated plots during the VE and V4 growth stages, but followed by significantly higher densities during R2 ($P = 0.017$). In accordance with the positive taxon weights, mean captures of scelionid wasps (Scelionidae), centipedes (Chilopoda), predaceous mites (Mesostigmata), fungivore beetles (see Table S1 in the Supporting Information, Coleoptera fungivores), millipedes (Diplopoda) and crickets (Gryllidae) were numerically lower by 20 to 38% in the insecticide treated plots but statistically not different from captures in the control plots. In contrast, the seed treatment had a positive effect on two major saprovore taxa – springtails (Collembola) and soil mites (Acari). Pooled over three springtail families (Sminthuridae, Entomobryidae, Isotomidae), pitfall captures during plant emergence (VE) averaged 232.9 ± 66.5 and 109.7 ± 26.6 in the insecticide treated and control plots, respectively, but overall abundance returned to similar levels in all plots during V4 and R2, as evidenced by a significant interaction effect ($F(_{2,35}) = 3.56$, $P = 0.039$). Likewise, soil mites (primarily belonging to the order Oribatida) were consistently more abundant (overall 23.3% higher) in the treated plots during all trapping periods, which was reflected by the negative taxon weight; however, ANOVA results showed non-significant treatment and interaction effects.

The overall effect of treatment on arthropod abundances was not significant for litter samples, but abundances were generally lower in the insecticide treated plots or showed only slight differences for the majority of taxonomic groups. Rove beetle abundance was 40.8% lower in the insecticide treated plots but the difference was not statistically significant. Only densities of ants were significantly reduced by the seed treatment ($F(_{1,3}) = 12.0$, $P = 0.040$). Although the relative composition of litter communities was similar to that of the pitfall captures, responses to the insecticide seed treatment differed noticeably for several major groups, particularly springtails and soil mites. The positive response in abundance of these arthropods that was clearly evident from the pitfall trap data was not reflected in the litter data.

3.2 Foliage-dwelling community

Pooled over sites, 30 arthropod taxa were identified by the visual plant inspections, of which 96% were herbivores (Table S1 in the Supporting Information). Overall, thrips (Thripidae), corn leaf aphids (*Rhopalosiphum spp.*), corn blotch leafminers (*Agromyza parvicornis* Loew), and corn flea beetles (*Chaetocnema pulicaria* Melsheimer) were the predominant plant feeders. Leafminer density was estimated by counting the number of individual mines in leaves, since visual counts of larvae or adults were not possible.

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Minute pirate bugs, [Anthocoridae, *Orius insidiosus* (Say)], spiders (Araneae), and ladybird beetle adults and larvae [Coccinellidae, mainly *Coleomegilla maculata* (DeGeer)] were the major beneficial organisms at both sites. Psocids (Psocidae), saprovorous beetles (Coleoptera, see Table S1 in the Supporting Information), and immature flies (Diptera) were the major saprovores found on plants but altogether accounted for less than 1% of the total abundance. Adult parasitic insects and flies (Diptera) were probably underestimated by plant inspections due to their mobility and small size. Pooled over sites, the total abundance of arthropods ($F_{(3,49)} = 3.82$, $P < 0.016$) was significantly lower in the insecticide treated plots, particularly during the V4 and V6 stages, with mean numbers per 25 plants of 128.9 ± 15.0 and 385.3 ± 77 in the treated and control plots, respectively (Table 1). However, the insecticide treated plots exhibited significantly greater diversity and taxa evenness as indicated by the higher Simpson diversity index ($F_{(1,1.86)} = 40.0$, $P = 0.029$).

The principal response curve in Figure 2 indicates that the foliage-dwelling community was significantly affected by the insecticide seed treatment. The first ordination axis explained 63.9% of the variance in the fitted data, and the Monte Carlo permutation test showed a significant negative community response in the insecticide treated plots over time ($P = 0.002$) compared to the control community. Departures in the overall abundances of taxa were greatest at V4 and V6, about 18 to 28 days after planting, and the disturbed community steadily recovered and was not significantly different in taxa abundances at anthesis (approximately 75 days later). Less abundant arthropod groups (highest positive weights) in the insecticide treated plots included: thrips (Thripidae), leaf blotch miners (Agromyzidae), fungivore beetles (primarily Phalacridae, Nitidulidae, and Corylophidae), spiders (Araneae), grass flies (Chloropidae), and minute pirate bugs (Anthocoridae). Only the general group of leafhoppers (Cicadellidae) showed a high negative taxon weight, indicating that these herbivores were more abundant in the seed treatment plots. Thirteen taxa with weights near zero are not listed in Fig. 2 because their affinity to the response curve was unrelated or too weak to interpret.

Analysis of individual arthropod groups revealed significant reduction in the overall abundances in insecticide treated plots for thrips (75.6% less; $F_{(1,6)} = 47.8$, $P < 0.001$), leaf miners (19.4% less; $F_{(1,2.9)} = 280.5$, $P < 0.001$), and minute pirate bugs (41.3% less; $F_{(1,6)} = 65.6$, $P < 0.001$). For most arthropod groups, the treatment by time interaction revealed no changes in direction of the treatment effect with sampling date. Differences in thrips abundance were the greatest during the V4 and V6 growth stages when immigrant thrips from nearby, senescing wheat fields colonized plots. The overall mean number of thrips per 25 plants during these growth stages averaged 39.5 ± 9.8 and 275.8 ± 84.0 in the treated and control plots, respectively. Differences in abundances of leaf miners and minute pirate bugs were most apparent during late whorl (V9), when the treatment by time effect was statistically significant for the minute pirate bug predators ($F_{(3,113)} = 5.5$, $P = 0.001$). It is noted that the principal response curve analysis generated a negative weight for leafhoppers, indicating greater abundance in the insecticide treated plots. This response was statistically significant for leafhoppers ($F^{1,6} = 6.4$, $P = 0.045$), with densities recorded in treated plots that were about double those in control plots

3.3 Aerial community

Sticky card sampling provided abundance estimates of 53 aerially active arthropod groups, many of which were underestimated or not recorded by plant inspections (Table S1 in the Supporting Information). Herbivores and saprovores comprised 55% and 29% of the aerial community, respectively, based on total numbers of individuals captured. Leafhoppers, thrips, flea beetles, aphids, grass flies and planthoppers (Delphacidae) were the most abundant plant feeders. Scuttle flies (Phoridae), dark-winged fungus flies (Sciaridae), and shining mold beetles (Phalacridae) represented the major saprophytic groups. Of the predators, which accounted for only 3.5% of the total captures, fireflies (Lampyridae), long-legged flies (Dolichopodidae), lady beetles (Coccinellidae), and minute pirate bugs (Anthocoridae) were the most abundant. Sticky cards were particularly informative for estimating densities of parasitic wasps, of which 15 families were recorded. Mymaridae, Scelionidae, Trichogrammatidae, and Braconidae accounted for 92% of the parasitoid complex. Mean total abundance and number of taxa per sticky card were slightly lower in the insecticide treated plots but not significantly different (Table 1). Taxa diversity and evenness were also not affected by the seed treatment.

The overall responses of arthropods aerially active in the plant canopy were similar to the plant-dwelling community, showing negative departures from the control at VE and V4, with a trend towards recovery at R2 (Fig. 3). According to the first axis of the principal response curve analysis, 69.0% of the variation in the aerial community was explained by the seed treatment by sampling time interaction effect, which significantly affected the population abundances of many taxa compared to the control community ($P = 0.002$). Eighteen arthropod groups with positive taxon weights had overall abundances that followed

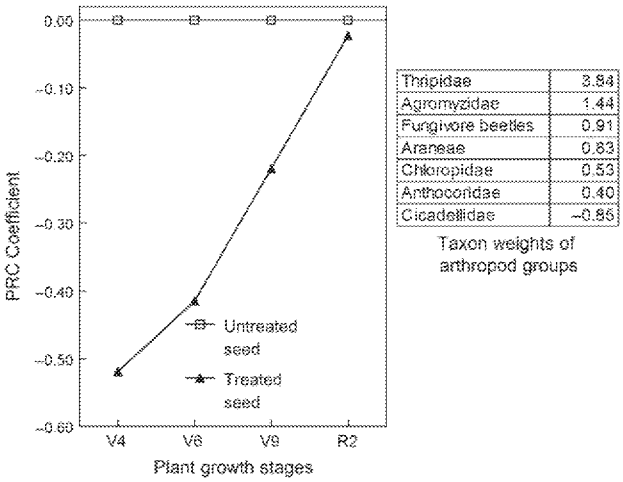

Figure 2. Principal response curve (PRC) showing the effects of clothianidin insecticide-treated corn seed for the foliage-dwelling arthropod community at seedling stage (V4), early (V6), late whorl (V9), and anthesis (R2). Analysis was performed on visual count data pooled as averages over both study sites. The first ordination axis explained 63.9% of the variance in the data, and overall community response in the insecticide-treated plots significantly deviated from the zero reference line representing the control community ($P = 0.002$). Higher positive weights indicate that arthropod abundances in the insecticide-treated seed plots followed the trend depicted by the response curve, whereas higher negative values inferred the opposite. Most affected arthropod groups include thrips (Thripidae), corn blotch leafminers (Agromyzidae), fungivore beetles (mainly Phalacridae, Nitidulidae, and Corylophidae), spiders (Araneae), grass flies (Chloropidae), minute pirate bugs (Anthocoridae), and leafhoppers (Cicadellidae). Thirteen taxa with weights near zero are not listed because their affinity to the response curve was unrelated or too weak to interpret.

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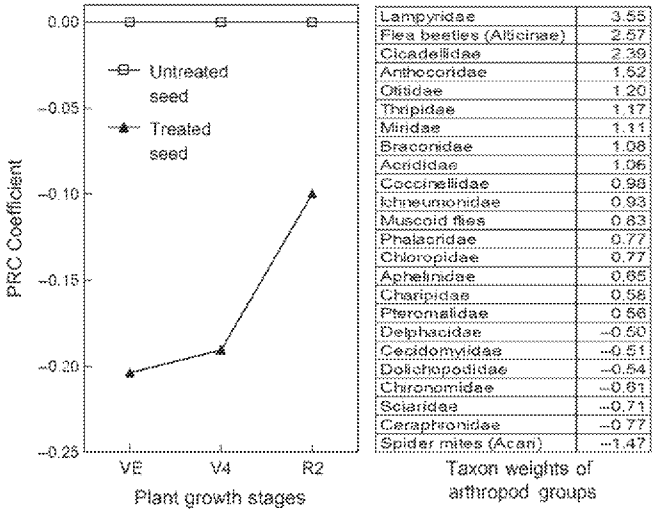

Figure 3. Principal response curve (PRC) showing the effects of clothianidin insecticide-treated corn seed for the aerially active arthropod community during plant emergence (VE), seedling stage (V4), and anthesis (R2). Analysis was performed on sticky card data pooled as averages over both study sites. The first ordination axis explained 69.0% of the variance in the data, and overall community response in the insecticide-treated plots significantly deviated from the zero reference line representing the control community ($P = 0.002$). Higher positive weights indicate that arthropod abundances in the insecticide-treated seed plots followed the trend depicted by the response curve, whereas higher negative values inferred the opposite. Most affected arthropod groups include fireflies (Lampyridae), flea beetles (*Chaetocnema pulicaria* Melsheimer), leafhoppers (Cicadellidae), minute pirate bugs (Anthocoridae), picture-winged flies (Otitidae, synonym Ulidiidae), thrips (Thripidae), plant bugs (Miridae), parasitic wasps (Braconidae, Ichneumonidae, Aphelinidae, Charipidae, Pteromalidae, Eulophidae, Ceraphronidae), grasshoppers (Acrididae), ladybird beetles (Coccinellidae), muscoid flies (Muscidae, Anthomyiidae, Sarcophagidae, Tachinidae), shining mold beetles (Phalacridae), grass flies (Chloropidae), crickets (Gryllidae), planthoppers (Delphacidae), gall midges (Cecidomyiidae), long-legged flies (Dolichopodidae), midges (Chironomidae), dark-winged fungus flies (Sciaridae), spider mites (Acari). Eighteen taxa with weights near zero are not listed because their affinity to the response curve was unrelated or too weak to interpret.

the trends depicted by the response curve, whereas the negative weights of eight taxa indicated the opposite, an increase in abundance.

ANOVA results showed significant adverse effects due to the insecticide seed treatment on the following arthropod populations: fireflies (70.4% reduction; $F_{(1,6)} = 7.0$, $P = 0.038$), flea beetles (46.2% reduction; $F_{(1,6)} = 46.8$, $P < 0.001$), leafhoppers (35.6% reduction; $F_{(1,6)} = 17.1$, $P = 0.006$), minute pirate bugs (66.2% reduction; $F_{(1,2.86)} = 96.9$, $P = 0.003$), lady beetles (44.7% reduction; $F_{(1,2.52)} = 37.7$, $P = 0.014$), grasshoppers (Acrididae, 38.8% reduction; $F_{(1,4.9)} = 10.2$, $P = 0.025$), and picture-winged flies (Ulidiidae, 31.7% reduction; $F_{(1,2.68)} = 36.2$, $P = 0.013$). Thrips, plant bugs (Miridae), grass flies, fungus beetles, and most hymenopteran parasitoid families (except ceraphronid and eulophid wasps) were also less abundant in the insecticide treated plots but differences were not statistically significant, either analyzed individually or as a trophic group. Of the arthropod groups with negative weights, all showed higher abundance levels in the insecticide treated plots but treatment differences were non-significant because mean counts of these taxa were too variable. In particular, mite captures on sticky cards were 52% higher in the insecticide treated plots but this response occurred mainly during plant emergence.

4 DISCUSSION

4.1 Impact on overall arthropod communities

This study assessed the community-level effects of clothianidin seed treatments on non-target arthropods in a mid-Atlantic no-till corn agroecosystem. A total of 72 219 arthropods, representing 118 families and 14 orders, were observed in the foliar and epigeal communities. Of the total arthropods recorded, 29.3%, 53.7%, 13.2%, and 3.7% were herbivores, saprovores, predators and parasitoids, respectively. With the exception of those extracted from litter, the arthropod communities differed between seed treatments that did and did not contain the clothianidin insecticide, with PRC analyses revealing both negative and positive changes in taxa abundances, primarily during the 4 weeks following planting. In general, arthropods on plants and those aerially active in the foliage were more drastically reduced by clothianidin than the epigeal arthropods. Community composition and diversity measurements varied by sampling method, with pitfall traps demonstrating lower epigeal arthropod diversity in the clothianidin-treated plots and visual samples exhibiting a significant increase in diversity and taxa evenness in the foliage-dwelling community as a result of the insecticide seed treatment. This increase in the visual samples likely occurred because the most abundant taxa, thrips, were significantly reduced by the systemic insecticide, thus resulting in more even composition of arthropod groups. Similarly, a community analysis of arthropods in anthesis stage corn in the Northern Great Plains, discovered a negative correlation between pest abundance and species diversity as well as community evenness, with fewer herbivores found in more even and diverse arthropod communities.[53]

All disturbed communities of arthropods showed consistent trends toward recovery at the later corn growth stages. At anthesis, abundances of arthropods were not significantly different from those observed in the control community. Arthropod communities also tend to be the most abundant and diverse at this stage in corn phenology.[53,54] The decline in non-target effects over time was likely linked to the decreasing titer of clothianidin in the plants. Recent work determined that clothianidin concentrations in plant tissue (root and seedling) decline rapidly in the first 20 days after planting, with less than 1.5% of the applied clothianidin being taken up by the plant.[55] Little is known about the environmental fate of the remaining 98% of the active ingredient; however, there is evidence that much of it is lost to ground and surface water.[43,55,56] Even shortly after planting, when the initial soil concentrations of clothianidin are the highest, studies have indicated a low risk to non-target invertebrates because the expected environmental soil concentrations are lower than the no-observed effect concentration for the most sensitive tested species. Plant titers of other neonicotinoids used as soil systemics at higher rates are also known to decline rapidly over time.[57–59]

4.2 Impact on herbivores

As a feeding guild, herbivores were more drastically affected than the other trophic groups due to their direct exposure to clothianidin by feeding on plant tissue. Several genera of thrips (*Anaphothrips, Frankliniella,* and *Microcephalica* spp.) were the most abundant herbivores, and their populations were reduced by 76%. These insects rarely reach economically damaging levels and thus are considered a resource prey that attracts predators into the corn agroecosystem early in the growing season (Dively GP, unpublished). Sticky card captures of leafhoppers ranked second in herbivore abundance and were reduced by 36% in the

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insecticide treated plots compared to the control plots. Contrary to the sticky card data, visual counts of leafhoppers on plants were significantly higher in the insecticide treated plots. Since these sucking insects are susceptible to neonicotinoids,[58,60] it is possible that they were sub-lethally intoxicated, which could have reduced their mobility and increased the chances of being counted during visual plant inspections.[61] Like thrips, leafhoppers rarely reach damaging levels on corn in the mid-Atlantic US. Other herbivores, including corn leaf aphids, corn blotch leafminers, corn flea beetles, and several species of plant bugs, were significantly reduced by 28 to 55% in the clothianidin-seed treated plots. The notion that the seed delivery of clothianidin may lead to pest outbreaks later in the growing season was not supported by this study. Aphids, lepidopteran larvae, and spider mite densities assessed by plant inspections and sticky cards during the entire sampling period were very low and did not indicate any overall trends that would suggest a treatment effect. Taken together, most herbivores observed in this study were not economically damaging to corn and thus probably had more beneficial value as prey for natural enemies. Therefore, any reduction in their populations could have bottom-up trophic effects on predators and parasitoids, particularly during the early season when it is important to attract beneficial arthropods into the corn agroecosystem.

4.3 Impact on saprovores

Saprophagous arthropods composed the majority of the epigeal community, of which soil mites and collembolans were by far the most abundant taxa. Pitfall traps during plant emergence recorded significantly higher abundances of these soil-dwelling arthropods in the insecticide treated plots. This response was unexpected because neonicotinoids and other insecticides are used in seed treatments to control these arthropods in several root and forage crops. When directly exposed to clothianidin in acute toxicity tests, survival of collembolans was reduced; however, the reproductive performance of the survivors was not adversely affected.[62] Several studies have reported population increases of collembolans resulting from a trophic cascade due to the negative insecticidal effects on predators.[63–65] Resurgence of phytophagous mites is also a common phenomenon due to disruption of their natural enemy populations after insecticide applications.[66,67] In this study, staphylinid and carabid populations in the surface litter were adversely affected by the clothianidin seed treatment, thus changes in collembolan and mite densities could be predator-mediated. However, litter extractions provided more absolute estimates of abundance and did not show any evidence of a seed treatment effect on these arthropods. Thus, the higher trap captures were more likely due to increased activity of springtails and soil mites on the soil surface, resulting from a possible repellency effect of the insecticide. Such a behavioral change in activity would increase the chances of capture in pitfall traps.

4.4 Impact on predators and parasitoids

Pooled together as a feeding guild, predators (primarily minute pirate bugs, ladybird beetles, lacewings, spiders, ground beetles, and rove beetles) in the epigeal and foliage communities were significantly less abundant in the insecticide treated plots by 20.2 and 34.5%, respectively. Other studies have reported similar negative effects on insect predators after exposure to neonicotinoid residues.[36,68–75] The predator composition and abundance in imidacloprid seed treated and untreated corn fields over a 5-year period showed adverse effects on Heteroptera, Staphylinidae, and to a lesser extent, Carabidae.[8] A germinated seed bioassay[73] found that clothianidin seed treatments adversely affected 16 carabid species. Six years of annual imidacloprid soil-applications in turf reduced adult staphylinid and carabid captures.[36] Our results agree with the findings of these studies and also provide evidence of even higher reductions (20.2 and 34.5%) in predator abundance than the levels (~16%) reported in the meta-analysis study.[21]

PRC results of the sticky card data also revealed positive taxon weights for several families of parasitic hymenopterans, indicating lower abundances by 19.4 to 60.9% in the insecticide treated plots. Although treatment differences were not statistically significant for the parasitoid taxa, consistent trends across multiple families provide some evidence of a possible adverse effect at this trophic level.

4.5 Routes of exposure

There are several exposure routes by which beneficial arthropods could come in direct contact with clothianidin seed treatments. Predators can be exposed to clothianidin by feeding on prey that has fed on the systemically treated plant.[24,74,76] This exposure route could also occur with parasitoid immatures *via* consumption of hosts that are feeding on plant tissue containing the toxicant.[57,75,77] Secondly, predators and parasitoids may directly consume contaminated plant material. Clothianidin seed treatments were particularly toxic to the more omnivorous carabid *Scarites quadriceps* Chaudoir compared to other carabids.[73] Certain sucking predators ingest systemic insecticides by feeding on the plant, such as *O. insidiosus*, which is known to probe plants and remove plant juices during periods of drought or limited prey.[78–80] Eggs can be directly exposed within the oviposition site, and *O. insidious* eggs placed in sunflowers *Helianthus annuus* L. grown from thiamethoxam-treated seeds exhibited reduced adult survival and fecundity.[15] Adult parasitoids can be directly exposed to the systemic insecticide by feeding on extrafloral nectar or guttation liquid, the latter of which is a common phenomenon during the seedling stages of corn. For example, sunflower extrafloral nectar reduced aphidiid wasp [*Lysiphlebus testaceipes* (Cresson)] parasitism and the proportion of female offspring when grown from thiamethoxam treated seed.[81] Beneficial arthropods may also be directly exposed to residues in the soil, especially epigeal organisms that spend parts of their life cycle within or on the soil.

There are also several indirect mechanisms by which beneficial arthropods can be affected by clothianidin seed treatments. As discussed previously, the lack of available prey or hosts during the first 4 weeks of corn growth probably delayed colonization and subsequent population buildup of many predators and parasitoids. When correlating arthropod abundance with predominant herbivores on corn plants at anthesis, aphid populations corresponded with numerical responses to multiple predators including coccinellids, anthocorids, spiders, syrphids, and carabids.[54] Likewise, thrips, aphids and cicadellids were the most prevalent herbivores in our study, and the foliar and aerial sampling data showed reductions in thrips that coincided with reductions in anthocorids. In some cases, exposure to neonicotinoids can also influence natural enemies' ability to find hosts. For example, soil applications of imidacloprid in early spring affected a tiphiid parasitoid's response to white grub frass trails and interfered with biological control.[82] Neonicotinoids can also impact natural enemy host finding by reducing odor cues. Herbivore-induced plant volatiles are used by natural enemies to locate prey or hosts.[57,83–85] Anthocorids, coccinellids and chrysopids are able to perceive and use

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odors from infested plants to find prey in natural conditions.[86–89] The early season management of above ground pests by neonicotinoid seed treatments reduces herbivore feeding injury and herbivore-induced plant volatiles. Thus, the absence of injured plants and herbivore prey in the clothianidin-treated plots could have reduced the release of important volatile stimuli for natural enemies, thereby attracting fewer predators and parasitoids to the plots. Any of these direct and indirect exposure mechanisms could affect beneficial arthropods associated with clothianidin-treated plants.

4.6 Limitations of the study and future research

This study was conducted over a single growing season in two no-till dryland corn fields in the mid-Atlantic, so results may be different in irrigated, conventional till systems in other production areas. Furthermore, we cannot draw any conclusions about the long-term ecological consequences of seed treatment disturbance of arthropod communities. Increasing acreage of other crops such as soybeans and small grains are also planted with neonicotinoid-treated seed.[2,3] The half-life of neonicotinoids in soil is highly variable, and given that seed treatments are used over consecutive years on multiple crops in rotation, there may be cumulative effects resulting from repeated usage of seed treatments.[91] It is therefore important to carefully monitor changes in potential pest insects and beneficial organisms that might result from the repeated use of neonicotinoid seed treatments and to conduct further field research to assess the potential long-term, ecological consequences of their use. Additionally, the majority of corn acreage in the US is now planted with seed treatments of clothianidin and other neonicotinoid insecticides at rates higher than the 0.25 mg a.i. per kernel rate evaluated in this study,[90] thus, our study likely underestimates effects even within a single season.

As our research question centered on ecological consequences, we did not record the impact of clothianidin seed treatments on corn yield, which would have provided further insight into whether the use of these treatments is economically justified. However, we observed low pest pressure throughout the study and previous research has found that neonicotinoid seed treatments do not provide yield benefits in corn in the absence of pest pressure.[92–95]

4.7 IPM framework

Because insecticide seed treatments are prophylactic, they are often considered to be incompatible with IPM.[7,90] Such preventative practices are appropriate in situations with a high likelihood of pest pressure, such as when planting into turf, or into a field that has multiyear pests like wireworms (Elateridae) that cannot be controlled using foliar insecticides,[96] or in fields where chronic early season pest pressure has been observed. However, insecticide seed treatments are often used in fields that do not face regular pest pressure, as insurance against occasional pests. Insecticide use in corn has increased from <50% of corn in the US being treated with insecticides from the 1950s to the 1990s, to 79–100% of corn being treated with neonicotinoid seed treatments by 2011, without a corresponding increase in pest pressure.[90] Even when targeting specific key pests, IPM practices can have greater economic benefits than preventive seed treatments. 'A study comparing prophylactic insecticide use (seed treatments and foliar sprays) to IPM practices (weekly scouting and targeted foliar sprays) for soybean aphid control found that while all three treatments were effective in preventing damage, the IPM treatment was the most cost-effective.[97] Unfortunately, due to the lack of availability of untreated corn seed, growers often have no choice but to use neonicotinoid seed treatments.[90] Corn seed lacking insecticide treatment needs to be made widely available to growers, so that insecticide seed treatments can be used only when economically warranted.

5 CONCLUSION

Clothianidin insecticide seed treatments disrupted arthropod communities in mid-Atlantic corn agroecosystems, with communities recovering over the season and becoming more similar to the fungicide seed treated control plots. The greatest reductions in arthropod captures occurred in early season herbivores, representing the expected management of pests. However, the thrips and other herbivores we observed would not be considered economically damaging pests in this production system. Instead, they provide early season food sources and plant-induced volatiles that should encourage natural enemy colonization of the field. Despite impacts on natural enemy abundances, the insecticide seed treatment did not result in pest outbreaks later in the growing season. Currently, many growers do not have the option of using untreated corn seed; however, whenever possible, decisions to plant insecticide treated seed should be made within an IPM framework.

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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