Research Article

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Preventative insecticides reduce seedling injury, but do not increase yield in Bt and non-Bt corn grown in the mid-Atlantic

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Abstract

BACKGROUND: Field corn production systems rely on preventative insect management tactics, including hybrids expressing plant-incorporated protectants that are treated with neonicotinoid seed treatments and sometimes in-furrow pyrethroids. While effective seedling pest control can be crucial because of the cost of replanting, these treatments target many of the same pests and may add unnecessary costs for growers. Furthermore, seedling pests in the Mid-Atlantic tend to be sporadic, and preventative insecticides may negatively impact natural enemies. To better understand the value of common preventative tactics, we evaluated pest pressure and compared a neonicotinoid seed treatment (clothianidin) and an in-furrow pyrethroid (bifenthrin) in *Bacillus thuringiensis* (Bt) and non-Bt corn hybrids.

RESULTS: In Bt hybrids, the in-furrow pyrethroid did not decrease pest injury, increase stand, or increase yield, while the neonicotinoid seed treatment decreased pest injury and increased stand but did not increase yield. In a non-Bt hybrid, both insecticides decreased pest injury, but neither increased stand or yield. Above- and below-ground pest injury was scarce throughout the study, but even in the site-year with the most extensive injury, insecticides did not result in yield gains.

CONCLUSION: Implementing efficient economically and environmentally sustainable corn pest management requires a thorough understanding of the contributions of each component of the pest control system. By thoroughly exploring pest pressure in Bt and non-Bt systems, this study shows that preventative insecticide use could be scaled back in many cases, especially given the environmental and economic costs associated with them.

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

Keywords: seedling insect pests; integrated pest management; neonicotinoid seed treatments; in-furrow pyrethroids; *Zea mays* (L.)

1 INTRODUCTION

Integrated pest management (IPM) has been an invaluable alternative to routine insecticide applications in agriculture since the mid-1900s, but has been weakened in recent decades by the widespread use of preventative management tactics, especially for early-season pests in field corn.[1–4] In an IPM framework, a variety of tactics are used to keep pests below economically damaging levels, usually reserving chemical controls for situations where the pest injury, determined through monitoring, will outweigh the cost of intervention.[5] This approach reduces insecticide use and decreases input costs, health risks for growers and consumers, and contamination in the environment.[5,6] While preventative treatments can be compatible with IPM when rescue treatments are not possible and pests are predictably damaging,[7] applying insecticides as 'insurance' when yield-limiting pest pressure is not expected does not follow IPM principles.[5] Nonetheless, preventative pest management for early-season pests, characterized as 'one size fits all,' has become widespread for field corn across the United States, regardless of pest pressure.[3,7–9]

The primary approaches that make up the 'one size fits all' early-season pest management package are corn hybrids with incorporated protectants sourced from *Bacillus thuringiensis* (Bt) bacteria and neonicotinoid seed treatments.[3,9] Pyrethroids, often as in-furrow formulas, may be applied in addition.[10,11] Bt corn expressing lepidopteran-targeting proteins was developed and introduced in 1996 in response to the serious stalk pest *Ostrinia nubialis* (Hübner) (European corn borer) and it continues to be largely effective in preventing damage from this pest in the United States.[12–14] Other Bt proteins were subsequently introduced, expanding Bt corn resistance to a range of lepidopteran and coleopteran pests occurring throughout the growing season.[3,15–17] Besides protection from caterpillar pests, widespread Bt use has also led to lower pest pressure in unprotected crops, including non-Bt corn.[18,19] Despite its insect pest control

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benefits, as Bt corn acreage has grown nationally, so has the use of neonicotinoid seed treatments and pyrethroid insecticides.[20] Neonicotinoid seed treatments are applied in increasing amounts to nearly all non-organic corn seed sold in the United States.[8,20,21] Neonicotinoids are systemic and seed treatments can protect the entire seedling,[22] although they only protect against early-season pests.[23] Above-ground pests targeted by neonicotinoid seed treatments include a host of pests from different orders: lepidopteran, coleopteran, and hemipteran pests that vary in their importance, but tend to be secondary pests.[22] Soil pest targets include *Diabrotica* spp. (western, northern, and southern corn rootworm), wireworms (Coleoptera: Elateridae), white grubs (Coleoptera: Scarabaeidae), and *Delia platura* (Meigen) (seed corn maggot).[22,24] At-planting pyrethroids can be used as a substitute or in addition to neonicotinoid seed treatments.[25] They target many of the same pests, especially pyrethroid-susceptible western corn rootworm populations in the Midwest,[24,26] and wireworms and white grubs elsewhere,[27,28] and additionally are marketed for lepidopteran pests in the armyworm species complex (Lepidoptera: Noctuidae) (https://ag.fmc.com/us/en/plant/capture-lfr-insecticide; https://www.syngenta-us.com/insecticides/force-6.5g). While most foliar pests can be managed with scouting and rescue treatments,[29] soil pests are hard to scout for, cannot be managed with rescue treatments, and may cause costly replanting, all factors that motivate and might justify preventative use.[11] By combining Bt hybrids, neonicotinoid seed treatments, and in-furrow pyrethroids, growers manage early-season above- and below-ground corn pests, although with little customization based on their regional or individual needs.[3,8]

In the US South and Corn Belt, at-planting insecticides provide pest control and yield benefits, but they can be inconsistent. In-furrow pyrethroids have sometimes reduced injury from western corn rootworms and annual white grubs, but did not increase yield.[27,30,31] Neonicotinoid seed treatments increase yield in continuous corn with western corn rootworm pressure,[32,33] but in highly replicated studies without specific pest pressure, yield benefits vary.[11,34] Preventative treatments may be even less consistently beneficial in Mid-Atlantic corn production. This region, which includes the states of Maryland, Delaware, Pennsylvania, Virginia, and West Virginia (https://www.epa.gov/aboutepa/epa-region-3-mid-atlantic), is characterized by small diversified farms.[35] Due to incentivization by conservation organizations, the Mid-Atlantic has some of the highest rates of reduced-tillage and cover-cropping practices,[36] and continuous corn which promotes corn rootworm and other soil pests is rare.[24] Due to a mix of growing practices and climate, many of the yield-limiting early-season pests that neonicotinoids successfully control do not occur in most parts of the Mid-Atlantic or occur only sporadically.[29] For example, neonicotinoid seed treatments sometimes improve yields when fields were infested with western and southern corn rootworm,[11,32,33,37] but these pests are scarce in the Mid-Atlantic outside of continuous corn fields.[9,29,38] Yield improvements have been linked to neonicotinoid seed treatments controlling several other pests, including chinch bugs (*Blissus* spp.) and various stink bugs,[10,39] but these pests do not or uncommonly occur during the seedling stage in most of the Mid-Atlantic.[29] The seedling corn pests that do occur in the Mid-Atlantic tend to be of mixed importance for growers and may not necessarily be best addressed with current management packages. Historically, caterpillars in the armyworm and cutworm species complexes were problematic for growers,[40] but they are no longer a main concern, likely due to a mix of Bt adoption, weed management, and neonicotinoid seed treatments.[15,29,41] Wireworms and white grubs can be damaging,[29,42,43] but even when these pests are above treatment thresholds neonicotinoids and in-furrow pyrethroids do not always improve yields.[27,28,43,44] For example, a high rate of neonicotinoid seed treatment improved yields in fields with above-threshold white grub pressure in only 1 year of a 3-year study in Virginia.[43] In fact, rather than any one insect pest, slugs tend to be the most concerning early-season pest for Mid-Atlantic growers.[45,46] Slugs are not controlled, and may even be exacerbated, by broad-spectrum insecticides.[46,47] The lack of consistently damaging insect pests leaves at-planting insecticides without an obvious target in the Mid-Atlantic.

Beyond regional variation, hybrid susceptibility is a driver of pest pressure that may influence the need for pest management. Although lepidopteran-traited Bt corn is widely planted, requirements for non-Bt refuge to manage resistance development paired with the higher cost of Bt seed[12,14,48] can lead growers to plant non-Bt corn. With different host-plant susceptibility, Bt and non-Bt corn constitute different production systems, but studies on the efficacy of at-planting insecticides have been concentrated largely in lepidopteran-targeting Bt corn[10,28,31,49] or in unspecified hybrids,[11,27,30] where resistance to a suite of caterpillar pests could mask insecticide efficacy. In managing corn rootworm species, neonicotinoid seed treatments helped protect non-Bt corn and corn containing ineffective Bt traits (due to evolved resistance) from injury,[33,50] suggesting treatments can be effective when they are not redundant. On the other hand, regional suppression of lepidopteran pests by Bt corn can drastically reduce pest pressure in non-Bt corn even though it lacks resistance.[18,19] Because most studies on the efficacy of at-planting insecticides pair treatments with Bt traits, this leaves pest pressure and insecticide performance in non-Bt systems understudied at a time when optimizing a low-input system could reap real benefits for growers.

Given that neonicotinoid seed treatments and in-furrow pyrethroids have inconsistent to negligible benefits in field corn throughout the United States, but have not been comprehensively tested under Mid-Atlantic pest pressure as well as in non-Bt systems, our goal was to test these strategies in both Bt and non-Bt corn systems across Maryland. We hypothesized that (i) low seedling pest pressure would lead to similar injury, seedling establishment, and ultimately yield in neonicotinoid seed-treated, in-furrow pyrethroid-treated, and untreated corn, and that (ii) if a difference between the treatments existed, it would be detected in the non-Bt hybrid. To test these hypotheses, we established a 3-year study across three University of Maryland research farms in which we compared early and season-long pest pressure, seedling stand, final stand, and yield in neonicotinoid seed treated, in-furrow pyrethroid treated, and untreated Bt and non-Bt corn hybrids. Because some claim that neonicotinoids can improve seedling vigor even in the absence of pests,[22,51] we also evaluated seedling growth. This study provides a thorough assessment of pest pressure and preventative insecticide performance in field corn and addresses a key knowledge gap in regional pest pressure.

2 METHODS

2.1 Study sites, field design, and treatments

To capture temporal and spatial variation in pest pressure and growing conditions, we replicated this study across three growing seasons (2020–2022) at three Maryland research farms: the

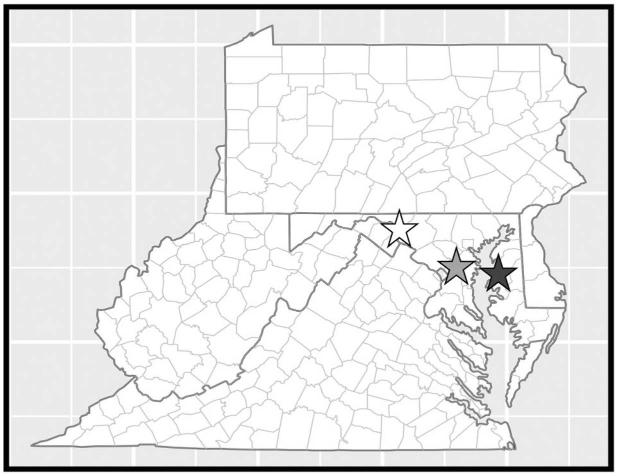

Figure 1. Study sites in relation to the Mid-Atlantic region. The white star represents the Keedysville research farm in Western Maryland, the light grey star represents the Beltsville research farm in central Maryland, and the dark-grey star represents the Queenstown research farm on Maryland's Eastern Shore.

Western Maryland Research and Education Center (Keedysville, MD), the Central Maryland Research and Education Center (Beltsville, MD), and the Wye Research and Education Center (Queenstown, MD) (Fig. 1). These three locations represent two of the four physiogeographic regions of the Mid-Atlantic, the Coastal region and Ridge and Valley region, and capture much of the climatic variation in the Mid-Atlantic.[52] The research farms have historically been managed using approaches typical of the region, including crop rotation, no-till, and cover-cropping. Each year, we planted corn as early as possible in keeping with local growing practices, although planting was delayed at two of three sites in 2022 due to wet weather (Table S1). Throughout the growing season, fields were maintained following standard fertility practices for non-irrigated no-till corn in the Mid-Atlantic (Table S2) and temperature and rainfall recorded (Table S3). In 2020, we planted plots of the Bt hybrid, LC1196 VT2P (Local Seed, Memphis, TN, USA), which expresses Cry1A.105 and Cry2Ab2 proteins, and in 2021 and 2022 we planted P1197YHR (Pioneer Hi-bred International, Inc., Johnston, IA, USA), which expresses Cry1Ab and Cry1F proteins. In all 3 years, the non-Bt variety we planted was the high-yielding hybrid, P1197LR (Pioneer Hi-bred International, Inc.). P1197 group hybrids are popular mid-season hybrids in Maryland, where they have high yields and yield stability, and are used as checks in variety trials.[53,54] LC1196 was chosen in a year when we were unable to source a P1197 Bt hybrid, so we used a hybrid with the same maturity group (111 days) and similar yield potential. We planted the Bt and non-Bt varieties in separate fields with three treatments: (i) bare, untreated seed with no insecticide applied in furrow, (ii) seed treated with Poncho® (Bayer Crop Science, Leverkusen, Germany), active ingredient clothianidin, at a rate of 0.25 mg per seed, and (3) bare seed with Capture LFR® (FMC Corporation, Philadelphia, PA, USA), active ingredient bifenthrin, applied in-furrow at the rate of 0.99 L ha^{-1}. The 0.25 mg per seed rate of clothianidin was typical for neonicotinoid seed treatments in the Mid-Atlantic in 2020, and chosen as a 'best case scenario' for non-target organisms, the focus of an additional study performed concurrently. The 0.99 L ha^{-1} rate of bifenthrin is specified by the label for wireworms, the pest we anticipated would cause the most injury. Across locations and years, we planted with a 74 100 seeds/ha rate. We replicated treatments three times in a complete Latin square (Fig. S1). Each replicate plot was a minimum of 61 m in length and 24 rows at 0.6 m spacing (18 m) in width.

2.2 Pheromone trapping lepidopteran pests

To assess the presence and activity of three migratory lepidopteran pests, we installed pheromone traps at each farm throughout the growing season. These traps targeted *Helicoverpa zea* (Boddie), corn earworm; *Spodoptera frugiperda* (J. E. Smith), fall armyworm; and *Striacosta albicosta* (Smith), western bean cutworm. To trap *H. zea* we used white plastic mesh Heliothis traps (Scentry Biologicals, Inc., Billings, MT, USA) baited with corn earworm lures (Scentry Biologicals, Inc.). In the top cone of the *H. zea* trap, one Vaportape II pesticide strip (Hercon Environmental, York, PA, USA) was safety pinned to the mesh to accelerate death of captured moths, reducing damage to specimens and facilitating collection. *Spodoptera frugiperda* and *S. albicosta* were monitored with green, white, and yellow and green-on-green universal bucket traps (Scentry Biologicals, Inc.), respectively. We used fall armyworm and western bean cutworm lures (Scentry Biologicals, Inc.), respectively, in the lure compartment for each trap, and Vaportape II pesticide strip in the bases. We checked all pheromone traps weekly, collected all moths, and froze samples until identification of target species.

2.3 Seedling establishment, pest injury, and growth

To evaluate the impact that the neonicotinoid seed treatment and in-furrow pyrethroid had on seedling stand and growth rate, we sampled plants in the V2–V4 growth stage 3–4 weeks after planting (Table S1) in all 3 years. In each plot we sampled three 15.2-m sections of rows, moving diagonally across the interior of the plots (~4.6 m apart). We counted the number of healthy plants (stand) and stunted plants. Plants were classified as stunted when they were estimated to be less than half the size of the average healthy plants, but without signs of pest injury. We counted the number of plants with injury classified in the following categories based on pests that are routinely present in Mid-Atlantic corn fields in our own and others'[55,56] previous work: (i) soil pest, (ii) cutworm, (iii) armyworm, (iv) slug, (v) stink bug, and (vi) miscellaneous foliar feeding injury (Fig. S2). Soil pest-injured plants were identified based on below-ground appearance. Plants with signs of soil pest injury, such as wilting center leaves,[57] were uprooted, and if identifiable feeding signs (holes in the below ground stem of the plant for wireworms or pruned root hairs for white grubs) or the pest itself was present, plants were categorized as injured by soil pests. We classified foliar pests based on the appearance of leaf feeding (Fig. S2). Cutworm injury was identified by either plants cut at ground level or mirrored holes in leaves resulting from the caterpillar boring into the whorl before the leaves unfolded.[57] While stinkbugs also cause mirrored damage, when caused by chewing mouthparts as with cutworm, the edges of the holes are crisp and undistorted. Billbug damage can also appear similar, and while we cannot entirely rule out billbugs, our previous work in field corn in Maryland and other work[29] suggests that this type of damage was likely predominantly caused by cutworm larvae, which more commonly occur in seedling corn. We classified the distinctive scalloped 'bites' along the margins of the leaves as indicating armyworms [primarily true armyworm (*Pseudaletia unipunctata*) and yellow-striped armyworm (*Spodoptera ornithogalli*), based on identification of larvae and because fall armyworm is rarely present in Maryland in the seedling stage].[57] Slug-injured plants

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showed distinctive streaks of 'window pane' feeding where slugs rasped the leaf tissue between veins.[46] We identified stinkbug injury by abnormal branching of seedlings where the growing point was destroyed or by elongated discolored holes where leaves were punctured while still in the whorl (edges of the holes were not missing upper epithelium, as seen in slug damage, and no clear scalloped margins, as seen with caterpillar chewing).[57,58] As mentioned above, billbug damage can be very similar but is much less likely. Finally, miscellaneous foliar injury was any feeding sign that did not fall into the previous categories, excluding physiological or mechanical leaf injury. Almost all physiological and mechanical injury was either 'buggy whipping,' where the tip of the seedling was stuck in the soil due to crusting, or wind injury, where the leaves were torn without any leaf area removed, as would be the case with most pest damage. Only one pest category, the one accounting for the greatest area affected, was recorded for each plant. Because soil pests typically killed entire plants, we counted these plants as soil pest-injured and did not record foliar feeding that occurred to them. Stunted plants were not included in pest injury sampling as they were already categorized based on a lack of injury. To assess the severity of foliar insect injury, in 2021 and 2022, we recorded the leaf area injured in cm^2 to the nearest 0.25 cm^2 using a transparent grid. To assess the growth of seedlings, in all years we measured plant height for 15 non-stunted seedlings from each replicate plot, five from each subsample location. We measured plant height from the soil to the tip of the extended longest leaf.

2.4 Pest injury in the mid- and late-season

To ascertain the mid- and late-season pest injury in Bt and non-Bt hybrids, we sampled plants targeting the V8–V9 and soft dough (R4) stages (Table S4). For the 2020 mid-season sampling, we sampled significantly after our target growth stage, with most locations being at R2, or the blister stage. We assessed one leaf per plant at a height of 1.4–1.7 m above the ground for injury from caterpillars, Japanese beetles, grasshoppers, piercing-sucking pests, leaf beetles (*Diabrotica* spp. adults), and leaf miners. Caterpillar injury included several different types: windowpaning from early instar fall armyworm, scalloped feeding on the leaf edge from true and yellow-striped armyworm, and rows of elongated holes from corn earworm and later instar fall armyworm feeding in the whorl before the leaves emerged.[57,59] Japanese beetle feeding was characterized by holes with a lacy appearance.[57] Grasshopper injury was usually in the form of irregular, often somewhat angular, holes or patches of leaf removal.[57,59] Piercing-sucking injury appeared as a row of small holes or discolored patches where pests punctured the leaf while it was in the whorl, as described for stink bug damage the seedling stage. Leaf beetle feeding took place as long irregular holes in the leaves.[57] Leaf miners caused irregular blotches between the upper and lower leaf surfaces.[57] We counted the number of plants with insect feeding and we measured the leaf area affected in cm^2 to the nearest 0.5 cm. We sampled 10 consecutive plants in three subsamples per replicate plot (rows 6, 12, and 18, moving diagonally through the plot). During the mid-season period in 2021 and 2022 we sampled pest injury prior to tassel emergence. While we timed our sampling to when the plants at a location had between 8 and 10 collared leaves visible, this number varied from 4 to 13 within locations. We assessed plants for similar injury as in 2020 (caterpillar, Japanese beetle, grasshopper, and stink bug injury), rating plants with a 5-point scale to assess degree of injury (Table S5), a measurement which accounted for the severity of caterpillar boring which was not captured in 2020 with leaf area measurements. We sampled 10 consecutive plants in four subsamples per replicate plot (rows 5, 10, 15, and 20, moving diagonally through the plot).

For ear sampling in all years, we assessed the ears from 10 consecutive healthy corn plants, skipping stunted plants (more detail below), in four locations per plot (rows 5, 10, 15, and 20, moving diagonally through the plot). At soft dough we removed ears from the stalk, husked them, recorded the number of injured ears, and counted the area of kernels affected in cm^2 using a transparent grid. Injury was classified as caused by caterpillar, stink bug, or sap beetle based on the characteristic feeding signs of each. Caterpillar injury, almost exclusively caused by *H. zea*, was evidenced by lines or patches of feeding where consecutive kernels were consumed, generally leaving the space filled with pale damp frass.[60] For sap beetle injury, kernels were hollowed out and there was often white, granular frass scattered across the injured area.[60] Stink bug injury manifested with two appearances: when severe, the entire ear was contorted into curved shape, and in less severe cases, the ear appeared normal externally, but individual kernels showed discolored starburst patterns from the piercing-sucking injury.[58,60]

2.5 Final stand and yield

To assess season-long stand loss, we measured the final stand at the end of each growing season and prior to harvest. As described in our seedling sampling, we measured the stand in three 15.2-m subsamples of rows moving diagonally across plots. In each subsample we counted the number of healthy and stunted plants. Plants that were less than half the diameter of the average healthy plants and that had formed no ears or half-sized ears were classified as stunted. When sufficiently dry (18–20% moisture), we harvested plots with a combine harvester and measured the yield from each plot using a calibrated combine, weigh wagon, or truck scale, depending on the equipment available at each farm. Subsamples of grain from each plot were collected and their moisture measured with benchtop grain moisture testers (Dickey-John, Auburn, IL, USA) at each of the farms. The total weight and percentage moisture for each plot were recorded, converted to bushels per acre corrected to 15.5% moisture, then converted to kg ha^{-1} using a weight of 25.4 kg per bushel.

2.6 Statistical analysis

Bt and non-Bt fields were analyzed separately for all outcome variables. For each variable, subsample data were pooled within each replicate plot, using different methods depending on the response variable. For seedling and final stands, stunted plants, as well as the number of insect-injured seedlings, whorl stage plants, and ears, counts were added across subsamples to give total counts per replicate plot. For injury, we performed analysis on counts, but present data as percentages for greater interpretability. Because of zero-inflation in each category of seedling pest injury due to frequent absences, we added categories together to give a total pest-injured seedling count for analysis. Total stand was converted to the number of plants per acre by dividing the total stand by the area sampled (375 ft^2), then multiplying by the number of feet per acre (43 560). The plants per hectare was then obtained by multiplying the plants per acre by 2.47. For stunted plants we used a simple total and did not convert to a per-hectare number because of the frequencies of zeros for this variable. For plant height and injury severity in the seedling, whorl, and ear stages, data were averaged across subsamples to give a mean value per replicate plot. Each outcome variable was analyzed individually using linear mixed models with the 'lme4' package in RStudio.[61] For all models, treatment was specified as the fixed effect and year and location were included as unnested random effects. For plant height, because the number of days after planting introduced substantial variability in the data, we included the days after planting as a random effect as well. For pest-injured seedling counts, we included an offset term, the log of stand count, to account for different numbers of plants that were sampled in each plot. In addition to the linear mixed models testing the effect of treatments on response variables over the 9 site-years, we additionally used linear models (function lm[62]) to test treatment effects at Beltsville in 2022 by itself. This was because we detected much higher wireworm pressure at Beltsville in this year and wanted to determine whether the treatments improved stand or yield under these conditions specifically. As linear mixed models are robust to non-normality,[63] we did not transform data when they failed assumptions of normality determined by a Shapiro–Wilk test. We tested heterogeneity of variance with Levene's test from the 'car' package.[64] Only one outcome variable, pest-injured seedling counts in the non-Bt hybrid, had heterogeneous variance, and it was addressed with a log (x + 0.5) transformation. When treatment significantly affected the response variable, we performed post-hoc tests with 'emmeans' using a Tukey HSD adjustment ($\alpha = 0.05$).[65]

3 RESULTS

3.1 Season-long monitoring of migratory lepidopteran pests

Of the three pests monitored with pheromone traps throughout the season, *H. zea* was the only one that was consistently present and the only species that occurred at Beltsville. While peaks of *H. zea* activity varied in their magnitude and timing between locations and years, for most locations in most years, peaks of activity occurred between mid-season and late-season (ear stage) sampling and after late-season sampling (Fig. S3). *Spodoptera frugiperda* and *S. albicosta* captures indicate that pest pressure was rare for both pests and did not overlap with vulnerable stages of the crop.

3.2 Seedling pest injury and insecticide impact

Total pest injury to untreated controls varied across site-years. On average, 7.06 ± 0.93% of Bt seedlings were injured, while 9.06 ± 1.57% of non-Bt seedlings were injured. Across site-years, this injury ranged from 2.51% to 12.95% of plants for the Bt hybrids. In the non-Bt hybrid, the injury ranged from 2.44% to 25.45%. The upper boundary of injury in the non-Bt hybrid was driven by armyworm feeding, which occurred to 22.87% of the seedlings sampled at Keedysville in 2020. Overall, armyworm injury in control plants occurred to 1.57 ± 0.49% of Bt seedlings and 3.70 ± 1.46% of non-Bt seedlings on average. Cutworms injured 3.22 ± 0.50% of Bt seedlings and 1.81 ± 0.26% of non-Bt seedlings. Non-lepidopteran foliar pest injury, which included stink bug feeding, is not controlled by Bt toxins. Control injury averaged 1.10 ± 0.28% of Bt plants and 0.63 ± 0.13% of non-Bt plants. Soil pests injured an average of 1.18 ± 0.45% of Bt seedlings and 2.92 ± 1.05% of non-Bt seedlings in the control treatment across the study. In 2022 at Beltsville, soil pest feeding occurred on more seedlings. Here, the mean injury in the control was 4.62 ± 2.08% for the Bt hybrid and 16.60 ± 1.83% for the non-Bt hybrid. Throughout the study, wireworms were the only soil organisms found feeding on plants with below-ground injury symptoms.

Insecticide treatments reduced total insect injury in the Bt hybrids ($F_{2,74} = 10.60$, $P < 0.01$) and non-Bt hybrid ($F_{2,74} = 16.08$, $P < 0.01$) (Fig. 2), especially the neonicotinoid seed treatment, which reduced the number of insect-injured seedlings by 64.73% in the Bt hybrids and 69.16% in the non-Bt hybrid compared to the control plots. The in-furrow pyrethroid treatment did not impact pest injury in the Bt hybrids, but lowered it by 60.14% in the non-Bt hybrid.

Neither insecticide treatment reduced feeding severity (cm^2 of leaf missing) in the Bt hybrids ($F_{2,44} = 2.31$, $P = 0.11$) or in the non-Bt hybrid ($F_{2,46} = 0.84$, $P = 0.44$) (Fig. S4). For both, the average leaf area affected per plant was ≤0.75 cm^2 in all treatments. The number of stunted plants was the same between treatments in both Bt and non-Bt hybrids at the beginning of the season ($F_{2,74} = 1.10$, $P = 0.34$, and $F_{2,74} = 0.71$, $P = 0.49$, respectively) (Fig. S5). In the Bt hybrids, on average 3.20 ± 0.30% of seedlings were stunted, while in the non-Bt hybrid 1.75 ± 0.17% were stunted.

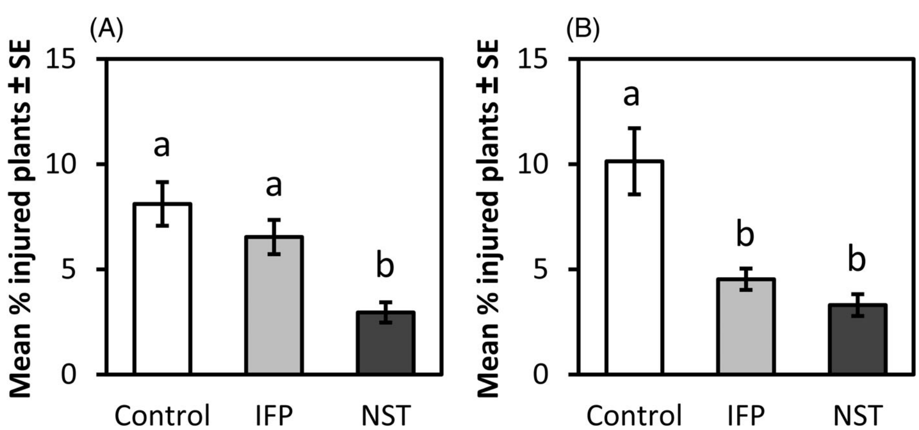

Figure 2. Mean percentage of seedlings injured by any insect pests (soil, lepidopteran, and non-lepidopteran combined) ± standard error (SE) in (A) Bt and (B) non-Bt hybrids for untreated control, in-furrow pyrethroid-treated (IFP), and neonicotinoid seed treatment (NST)-treated field corn across 3 years and three locations. Statistical analysis was performed on the corresponding count data using a linear mixed model specifying year and location as random effects and including an offset of the log of plant stand. Within panels, bars that have different letters are significantly different by Tukey's test ($\alpha = 0.05$).

3.3 Seedling establishment and growth

At-planting insecticides impacted seedling stand in the Bt hybrids ($F_{2,74} = 3.60$, $P = 0.03$), with the neonicotinoid seed treatment increasing seedling stand for Bt hybrids 8.19% over the untreated control, from 58 375 plants per hectare to 63 584 plants per hectare on average. However, the in-furrow pyrethroid treatment did not affect stand for Bt hybrids, and neither insecticide increased stand in the non-Bt hybrid ($F_{2,74} = 1.35$, $P = 0.27$) (Fig. 3). For Beltsville 2022, which experienced heavy wireworm pressure, insecticides impacted stand in both Bt and non-Bt hybrids (Bt: $F_{2,6} = 8.14$, $P = 0.02$; non-Bt: $F_{2,6} = 5.09$, $P = 0.05$) (Fig. S6). The in-furrow pyrethroid increased stand 44.42% compared to the control in the Bt hybrid and 15.18% in the non-Bt hybrid. The neonicotinoid seed treatment increased stand 46.87% in the Bt hybrid, but did not increase stand in the non-Bt hybrid.

Bt seedling height was affected by at-planting insecticides ($F_{2,68} = 5.37$, $P < 0.01$). Seedlings treated with the in-furrow pyrethroid or neonicotinoid seed treatment were 5.70% and 7.44% taller than untreated control corn, although this difference was only 1.82 cm on average (Fig. 4). Non-Bt seedling height was the same across treatments ($F_{2,70} = 0.50$, $P = 0.61$).

3.4 Mid-season pest injury

Insect injury in the mid-season (R2 and whorl stages) was rare in 2021 and 2022, and always of low severity. In 2020, foliar injury measured at R2 occurred to 20.86 ± 2.34% of Bt plants and 18.02 ± 1.78% of non-Bt plants across treatments, but in both hybrids the severity of this injury averaged <0.50 cm^2 per sampled leaf. Injury incidence was measured at the whorl stage in 2021 and 2022, and at this stage, only 3.43 ± 0.58% of Bt plants and 6.99 ± 1.04% of non-Bt plants had signs of feeding. Nearly all injury was rated at the lowest level (2, minor feeding). Only six plants out of the 4 320 plants evaluated across 2021 and 2022 exceeded the lowest category, all in the non-Bt hybrid, and all falling into level 3, 'significant feeding, but stalk intact'. Mid-season injury was not affected by treatment in 2020 in terms of incidence (Bt: $F_{2,22} = 0.11$, $P = 0.89$; non-Bt: $F_{2,22} = 0.40$, $P = 0.67$) or severity (Bt: $F_{2,22} = 2.60$, $P = 0.10$; non-Bt: $F_{2,22} = 0.85$, $P = 0.44$). Similarly, in 2021 and 2022 treatment had no impact on injury incidence in the whorl stage ($F_{2,48} = 2.22$, $P = 0.12$ in Bt, and $F_{2,48} = 0.58$, $P = 0.57$ in non-Bt). Severity was not analyzed in 2021 and 2022 due to the low incidence of damage.

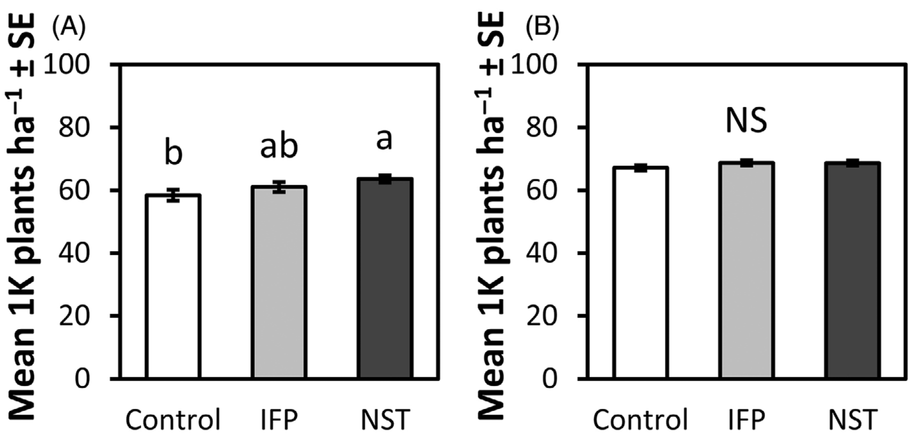

Figure 3. Mean seedling stand in thousand (1 K) plants ha^{-1} ± standard error (SE) in (A) Bt and (B) non-Bt hybrids for untreated control, in-furrow pyrethroid-treated (IFP), and neonicotinoid seed treatment-treated (NST) field corn across 3 years and three locations. Seedling number was compared between treatments using a linear mixed model specifying year and location as random effects. Within panels, bars that have different letters are significantly different by Tukey's test ($\alpha = 0.05$) and NS indicates no difference between treatments.

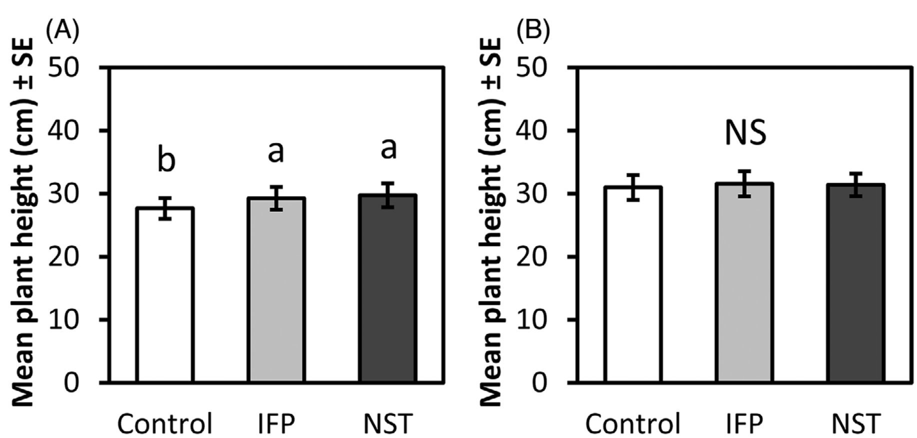

Figure 4. Mean V2–V4 plant height (cm) ± standard error (SE) in (A) Bt and (B) non-Bt seedlings for untreated control, in-furrow pyrethroid-treated (IFP), and neonicotinoid seed treatment-treated (NST) field corn across 3 years and three locations. Height was compared between treatments using a linear mixed model specifying year, location, and the number of days after planting as random effects. Within panels, bars that have different letters are significantly different by Tukey's test ($\alpha = 0.05$) and NS indicates no difference between treatments.

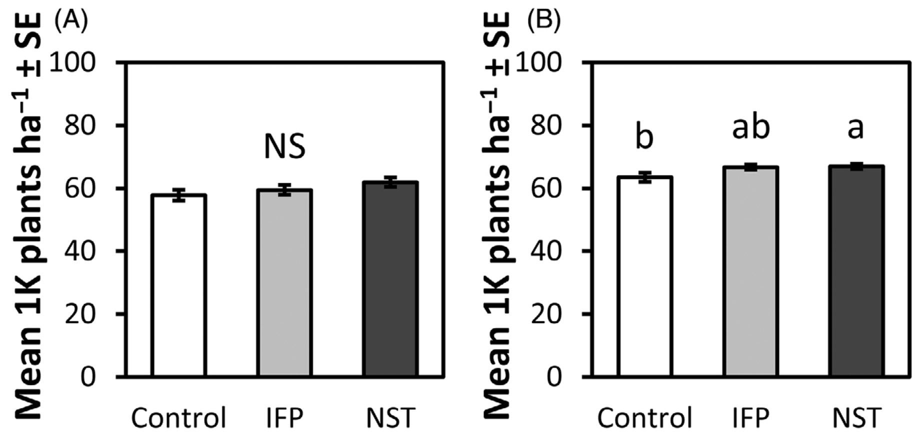

Figure 5. Mean end of season stand in thousand (1 K) plants ha^{-1} ± standard error (SE) in (A) Bt and (B) non-Bt hybrids for untreated control, in-furrow pyrethroid-treated (IFP), and neonicotinoid seed treatment-treated (NST) field corn across 3 years and three locations. Final stand counts were analyzed between treatments by linear mixed model specifying year and location as random effects. Within panels, bars that have different letters are significantly different by Tukey's test ($\alpha = 0.05$) and NS indicates no difference between treatments.

3.5 Late-season pest injury

The only lepidopteran pest that was found infesting ears was *Helicoverpa zea*. Across locations and years, feeding by *H. zea* ranged from 3.33% to 75.83% of Bt ears, with an average of 18.27 ± 2.47% ears injured overall. In the non-Bt hybrid, injury ranged from 4.17% to 83.33% of ears, averaging 20.71 ± 2.67% of ears. The area consumed per ear by *H. zea* was about ≤2 cm^2 in all site-years for both hybrids, except for Beltsville in 2022, where the feeding area for Bt was 8.93 ± 0.77 cm^2 per ear and for non-Bt was 9.04 ± 0.70 cm^2 per ear. This site-year also exhibited the most *H. zea*-injured ears in both corn hybrids. Stink bugs consistently injured ears. In the Bt hybrids, stink bug injury ranged from 2.78% to 71.94% of ears, and averaged 26.02 ± 3.00%, while feeding in the non-Bt hybrid ranged from 7.50% to 98.33% and averaged 46.82 ± 3.57% of ears. Stink bug injury in both hybrids was always <3 cm^2 per ear with the exception of the non-Bt hybrid at Queenstown in 2021 and 2022 where 7.10 ± 0.41 and 8.23 ± 0.38 cm^2 per ear was injured, respectively. Indeed, the greatest stinkbug feeding incidence and severity was found at Queenstown. Sap beetle incidence ranged from 0.56% to 46.94% of Bt ears with an average of 12.07 ± 1.67%, and 7.78 to 51.67% of non-Bt ears, averaging 21.02 ± 1.73%. Sap beetle feeding never exceeded 3 cm^2 per ear in either hybrid. Insecticide treatments did not alter the incidence or severity of *H. zea*, stink bug, or sap beetle injury to ears in Bt or non-Bt hybrids (incidence: Tables S6 and S7; severity: Tables S8 and S9).

3.6 Final stand and yield

At the end of the season, stand was similar between treatments in the Bt hybrids (Fig. 5(A); $F_{2,74} = 2.34$, $P = 0.10$), but differed in the non-Bt hybrid (Fig. 5(B); $F_{2,74} = 3.46$, $P = 0.04$), where the neonicotinoid seed treatment had 5.39% higher stand than the control. At Beltsville in 2022, treatments did not influence final stand in the Bt hybrids ($F_{2,6} = 3.91$, $P = 0.08$), but did affect stand in the non-Bt hybrid ($F_{2,6} = 24.36$, $P < 0.01$) (Fig. S7). Across the study, the number of stunted plants was the same across treatments in both the Bt (Fig. S8(A); $F_{2,74} = 0.56$, $P = 0.57$) and non-Bt hybrids (Fig. S8(B); $F_{2,74} = 2.91$, $P = 0.06$).

Although there were differences in pest injury, seedling and end of season stand, and height in one or both hybrids, treatment did not impact yield in either Bt or non-Bt hybrids ($F_{2,73} = 0.39$, $P = 0.68$, and $F_{2,74} = 0.22$, $P = 0.80$, respectively) (Fig. 6). The mean yield across years, locations, and treatments was 10 184 ± 235 kg ha^{-1} for Bt and 9 993 ± 246 kg ha^{-1} for non-Bt. Because of high percentages of soil pest-injured control plants and corresponding stand reductions at Beltsville in 2022, we also compared yields between treatments at that site-year alone. Despite the soil pest injury and stand loss, the untreated plots yielded as well as those with insecticide treatments for both Bt and non-Bt hybrids ($F_{2,6} = 0.67$, $P = 0.54$ and $F_{2,6} = 1.75$, $P = 0.25$, respectively) (Fig. S9). However, yields were notably lower and more variable at Beltsville in 2022, as well as at Beltsville in 2020 when the same fields were used, compared to the other site years (Table S10).

4 DISCUSSION

The goal of this study was to evaluate widely used preventative pest management in the context of season-long pest pressure in Bt and non-Bt corn systems in the Mid-Atlantic. Throughout this study, we rarely observed significant injury from the specific pests targeted by the preventative pest management strategies, and likely due to this low pest pressure we did not detect yield differences in either the Bt or non-Bt hybrids. At the seedling stage, consistent, but usually minor, injury occurred across most site-years for the pest types we evaluated (armyworms, cutworms, non-lepidopteran foliar pests, and soil pests). Non-lepidopteran foliar pests never exceeded 5% injury in Bt or non-Bt hybrids. Soil pests varied by location, occurring most frequently at Beltsville in 2021 and 2022, while being almost nonexistent at Keedysville in all years. In studies in Virginia, soil pests were similarly scarce and patchy.[28,42] Cutworms were more consistent, occurring in every site-year, but the injury incidence did not reach rule of thumb thresholds that growers might use when making a rescue treatment (5% for cutworms),[66] and was less frequent than reported in two out of 3 years in a Pennsylvania study.[67] Armyworm injury was absent in 2 out of 9 site-years for both Bt and non-Bt hybrids. Even at 1 site year with high armyworm feeding incidence (about 22% of non-Bt plants with injury), feeding did not reach rule of thumb treatment thresholds (25% of seedlings injured). The temporal and spatial variability of all of the insect pests we sampled agreed with previous reports that most seedling corn pests do not occur consistently,[7,29] especially in the

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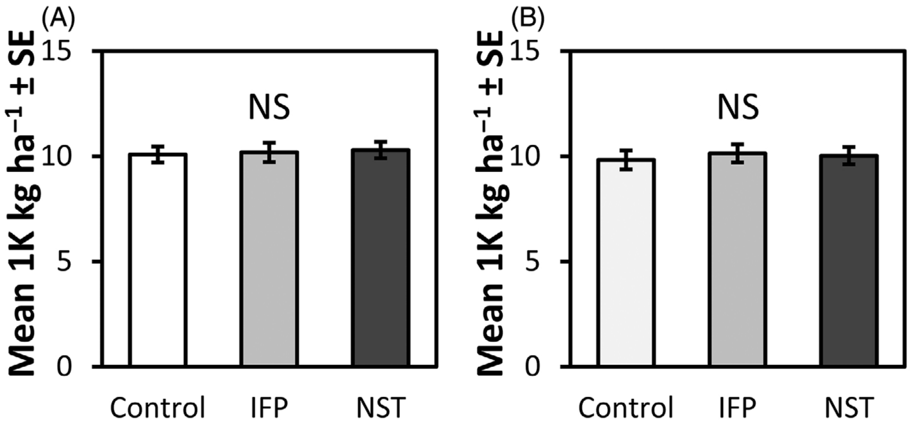

Figure 6. Mean yield in thousand (1 K) kg ha^{-1} yield ± standard error (SE) in (A) Bt and (B) non-Bt hybrids for untreated control, in-furrow pyrethroid-treated (IFP), and neonicotinoid seed treatment-treated (NST) field corn across 3 years and three locations. Yield was compared between treatments using a linear mixed model specifying year and location as random effects. There were no differences between treatments ($\alpha = 0.05$) and NS indicates no difference between treatments.

Mid-Atlantic.[49,68] Furthermore, when foliar injury occurred, the severity that we observed tended to be minor. For insect foliar pests, the mean amount of leaf area lost per V2–V4 seedling was less than 0.75 cm^2 across treatments in both Bt and non-Bt hybrids. As seedlings can be cut off at ground level up to V4 without yield loss,[69–71] the level of foliar injury we measured was unlikely to reduce yields.

Pest injury was also minor in the mid- and late-seasons. Prior to the introduction of Bt traits, whorl stage corn was prone to injury from *O. nubialis*,[72] however, we did not find economically important mid-season pests in either Bt or non-Bt hybrids. This contrasts with recent work from Pennsylvania, where *S. frugiperda* infested vegetative non-Bt corn, although that infestation varied by year.[45] Our pheromone traps showed that *S. frugiperda* was extremely rare during the growing season in all years, appearing in large numbers only late in the season in 1 year, consistent with *S. frugiperda* overwintering and migration.[73] In the ear stage, *H. zea*, stink bugs, and sap beetles caused injury. Roughly one fifth of ears in both hybrids (18.2% of Bt and 20.7% of non-Bt) were injured by *H. zea*. Sampling from 2010 to 2012 at many of the same research farms as our study found approximately 27.5% lower *H. zea* feeding in non-Bt hybrids than in our study.[12] The severity of this feeding to individual ears was lower than in our study as well. The greater injury we measured may have come from the widespread resistance that *H. zea* has developed to most Bt traits.[13,14]

Besides *H. zea*, stink bugs and sap beetles also consistently injured ears across site-years. In a recent survey in North and South Carolina, growers attributed the most yield loss in corn to *H. zea* and stink bugs,[74] and our findings show that they are ubiquitous ear pests in the Mid-Atlantic as well. While none of the pests that we observed in mid- and late-season corn are targets of at-planting insecticides, and it is unsurprising that the insecticides did not affect them, the lack of differences between treatments also showed that there were no secondary insect pest outbreaks associated with the insecticides.

In the absence of pest pressure, neonicotinoids have been credited with increasing the vigor and tolerance of abiotic stressors for a wide variety of crops,[22,51] and while seedlings were taller in both insecticide treatments in the Bt hybrids, it is unlikely that this phenomenon took place in our study for two reasons. First, while pests were below threshold, they were never entirely absent, and second, the in-furrow pyrethroid also increased height. These factors indicate that height differences likely came from reductions in subeconomic pest injury. As in another Maryland study,[49] the increase in height was not associated with higher yield, suggesting that reduced height in untreated corn, along with seedling foliar injury and reductions in stand mentioned above, is not always indicative of poorer eventual yield. Still, as plant height can be a significant indicator of yield,[75] more research is needed to determine whether at-planting insecticide-associated plant height improvements could have meaningful yield impacts.

While we did not detect significant yield improvements associated with the treatment-driven differences in insect injury, stand, and plant height, our results do not mean that at-planting insecticides are never a valuable tool for managing pests. At Beltsville in 2022, we had heavy wireworm pressure that was associated with greater than 40% reductions in seedling stand in the control Bt corn compared to either insecticide. This stand difference ultimately did not reduce yield, and while corn plants are able to compensate to some degree when establishment is reduced,[76,77] it is likely that our lack of yield difference was due to our specific insecticide treatments not providing sufficient long-term protection. The seedling stand improvements in the insecticide treatments were absent at the end of the season because of stand loss in the insecticide treatments in the Bt hybrid. Because we did not detect substantial mid-season pressure, this was probably due to seedling injury that occurred after our V3 sampling. This could have been because our rates were too low to protect seedlings through their entire period of vulnerability, or because the products were ineffective for another reason. For the in-furrow pyrethroid, we used the highest application rate (0.99 L ha^{-1}) that was recommended for wireworm when we initiated our study in 2020 because wireworm was the pest we predicted would most likely be yield-limiting based on previous studies at the research farms. When similar or higher rates were used as in our study (0.93, 0.98, and 1.23 L ha^{-1}), they also did not increase yield.[28,31] In contrast, there is evidence that our neonicotinoid rate was too low to control soil pests. In some studies where neonicotinoid seed treatments were tested in fields with white grub or western corn rootworm pressure, higher rates (1.25 mg per seed) improved yield while lower rates

(0.25 mg per seed) did not,[27,43,78] suggesting a higher rate is needed for soil pest control. Still, in another study with above-threshold wireworm pressure, even the 1.25 mg per seed rate of clothianidin only protected seedlings for about a month after planting, stand protection was lost by 6 weeks, and there were no yield benefits to using at-planting insecticides,[28] suggesting limited protection even at high rates. Significantly more studies are certainly needed to determine if high rates of bifenthrin or clothianidin can manage above-threshold wireworm pressure and improve yield. However, even if these treatments produced consistent yield benefits under pressure, it would still not mean that it pays off to use at-planting insecticides by default. An important aspect of wireworms and many white grub species is that these pests have multi-year lifecycles and persist multiple years, making damage predictable[29,43] and allowing at-planting insecticides to be targeted specifically to when and where soil pests are present.

Finally, the lack of yield differences at Beltsville in 2022 may simply have been due to poor performance in all of the treatments due to field conditions. Beltsville generally had lower and more variable yields than the other locations, and in 2020 and 2022 when our study was planted in the same field, yields were particularly low and variable. This field was poorly drained and prone to early-season flooding, and despite typical fertility management and normal weather conditions throughout the study (Table S3), never performed as well as other locations. It is possible that under conditions with better yield potential the seedling stand protection from the insecticide treatments would have improved yield. Indeed, in the non-Bt hybrid, the in-furrow pyrethroid slightly improved seedling stand, and both insecticides significantly improved final stand compared to the control. However, we were unable to detect differences in yield.

A potential limitation of our study is the number of sites-years over which we studied pest pressure and yield. Our 9 site-years cannot capture the full fluctuation of pest pressure across the Mid-Atlantic region over time, especially taking into consideration the unique conditions that might occur on grower farms based on their practices and geography. For example, Mid-Atlantic and Northeast growers who do not rotate their crops can build up yield-limiting pests and may benefit from at-planting insecticides.[4,38,78] Although the practices on our research farms (crop rotation, no-till, cover-cropping, and neonicotinoid use) are representative of much of the region,[20,35,36] they are not universally used. Additionally, while some insect pests tend to be predictable, like soil pests,[29] others may fluctuate less predictably and perhaps failed to have economic outbreaks during the 3 years of our study. Another challenge is that Maryland rarely has economic populations of some of the pests which are targeted by at-planting insecticides in the southern part of the Mid-Atlantic, namely chinch bugs and stink bugs.[10,11] To better understand the risk of yield-limiting early-season injury in untreated corn, multi-year and multi-site studies should be conducted throughout the Mid-Atlantic.

IPM relies on using chemical interventions judiciously, starting with best management practices to limit pest risks, and reserving applications for cases where pest injury is likely to result in yield losses that will outweigh any economic and environmental costs of their use.[3,5,79] Our study did not reveal yield benefits to outweigh the costs of these insecticides. Although a diverse complex of early-season pests attacked Bt and non-Bt seedlings throughout the 9 site-years of this study, plants were robust to injury and stand loss, and completely unprotected corn performed well. While at-planting insecticides are an important tool when there is a specific pest problem to target, these results highlight a mismatch between 'one size fits all' early-season pest control and the needs of many Mid-Atlantic corn growers, even when growing non-Bt corn. To fit within an IPM framework and realize the benefits of reduced inputs, pest management strategies should be brought into alignment with the pressure growers actually face, primarily by reserving their use for cases of above-threshold soil pest pressure.

However, customizing pest control is not an easy matter for many growers due to internal and external barriers. Even when growers understand and value IPM, many still use preventative management tactics based on perceptions of higher yield potential that are tied to industry recommendations.[74] A perception of risk can also keep growers from forgoing insecticide applications, especially when the cost of the insecticide is low.[80] Pyrethroids are notably inexpensive[81] and in-furrow application keeps the cost in time and effort low as well, so growers may apply pyrethroids 'just in case'. Like pyrethroids, neonicotinoid seed treatments may also be perceived as inexpensive, as their price is often obscured by conflation with the cost of fungicide seed treatments and the hybrid itself, which are generally bundled during purchase.[21,82] However, economic analyses show that neonicotinoid seed treatments do have concrete costs (\$16.80 ha^{-1} in 2018 and \$20.81 ha^{-1} in 2020 for a 0.25 mg ai per seed^{-1} rate),[11,83] even if they are not transparent to growers. Unlike pyrethroids, neonicotinoid seed treatments require significant advanced planning to opt out of and untreated seed may simply not be available to growers for the hybrids they wish to plant, forcing many growers to accept the costs of neonicotinoid seed treatments whether or not they are doing so intentionally.[8,82] Bt hybrids may be used by default for a similar reason, simply because few non-Bt hybrids are available,[74] even if they would be less expensive.

Besides the purchase costs associated with neonicotinoids and in-furrow pyrethroids, growers may benefit from reducing their environmental costs. Neonicotinoid seed treatments have widely demonstrated environmental costs to agricultural ecosystems where they can disrupt beneficial non-target insects such as detritivores,[84] pollinators,[85,86] and natural enemies,[49,68,87] and cause outbreaks of non-target pests.[47,88] In-furrow pyrethroids are less widely studied, but granular soil applications negatively affect non-target arthropods, including important predators.[84,89] While we did not observe secondary pest outbreaks, avoiding the well-established risks to natural enemies may help growers avoid subsequent pest problems.

Finally, our study suggests that Mid-Atlantic growers who eliminate all insect management inputs by growing completely untreated non-Bt hybrids may not face any major pest issues. Nevertheless, while this was true across our study, it is key to remember several details of our production system. First, our non-Bt corn was grown in a time and place where over 80% of corn contains Bt traits[14] conferring areawide suppression of key lepidopteran pests that could otherwise attack susceptible crops.[19] Additionally, our study was replicated on research farms that consistently use rotation and cover cropping, manage weeds, and have planted neonicotinoid-treated corn seed for decades. All of these factors play a role in reducing on-farm and regional pest pressure.[41,90–92] Completely eliminating Bt hybrids and at-planting insecticides might result in the reemergence of pest pressure, especially if IPM practices like crop rotation are not followed. As demonstrated in a 4-year study where *D. virgifera virgifera* began causing injury to continuous untreated non-Bt corn in year four: when no management steps are taken, pest pressure is likely to eventually reemerge.[4] Therefore, a wiser take-home message from our study is that pest pressure is likely to be changing and dynamic, and the best pest management strategy will follow the IPM tactics of using multiple non-chemical prevention strategies while monitoring pest pressure.

5 CONCLUSION

Preventative at-planting insecticide treatments did not increase yields in Mid-Atlantic corn across multiple locations and growing seasons for either Bt or non-Bt hybrids. While fewer plants were injured by insect pests in both insecticide treatments in the non-Bt hybrid, and in the neonicotinoid seed treatment in Bt hybrids, this injury was typically below economic thresholds with little leaf area removed. In Bt hybrids, insecticide treatments were also associated with small increases in stand and plant height, but these growth improvements were not accompanied by higher yields. These results, in combination with other studies failing to find consistent economic early-season pest pressure in Pennsylvania[67,84] and Maryland,[49,68] show that corn production in the Mid-Atlantic generally does not experience sufficient seedling pest pressure to justify routine use of preventative treatments. This, along with the widely demonstrated impacts of pyrethroids and neonicotinoid seed treatments on non-target organisms that fill a plethora of functional roles, suggests that growers should carefully consider their use of preventative insecticides within an IPM framework. Despite the challenge of obtaining untreated seed, saving money on unnecessary chemicals and potentially improving agricultural ecosystem services likely justifies the effort. To reduce the barriers to these efforts, untreated seed should be more available, so that growers can tailor their pest management system to the pest pressure in their region and on their farm.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Dryad at https://doi.org/10.5061/dryad.mcvdnck9h.

SUPPORTING INFORMATION

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

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