Helicoverpa zea (Lepidoptera: Noctuidae) feeding incidence and survival on Bt maize in relation to maize in the landscape

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Abstract

Background: Characterizing Helicoverpa zea (Boddie) damage to maize (Zea mays L.) in relation to the spatiotemporal composition of Bt crops is essential to understand how landscape composition affects H. zea abundance. To examine this relationship, paired Bt (expressing Cry1A.105 + Cry2Ab2) and non-Bt maize plots were sampled across North and South Carolina during 2017–2019. Kernel damage and larval exit holes were measured following larval development. To understand how maize abundance surrounding sample sites related to feeding damage and larval development, we quantified maize abundance in a 1 km buffer surrounding the sample site and examined the relationship between local maize abundance and kernel damage and larval exit holes.

Results: Across the years and locations, damage in Bt maize was widespread but significantly lower than in non-Bt maize, indicating that despite the widespread occurrence of resistance to Cry toxins in maize, Bt maize still provides a measurable reduction in damage. There were negative relationships between kernel injury and ears with larval exit holes in both Bt and non-Bt maize and the proportion of maize in the landscape during the current year.

Conclusion: Despite the widespread occurrence of resistance to Cry toxins in maize, this resistance is incomplete, and on average Bt maize continues to provide a measurable reduction in damage. We interpret the negative relationship between abundance of maize within 1 km of the sample location and maize infestation levels, as measured by kernel damage and larval exit holes, to reflect dispersion of the ovipositing moth population over available maize within the local landscape.

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

Keywords: Bt resistance; GIS; pest dilution; Helicoverpa zea; maize

1 INTRODUCTION

Helicoverpa zea (Boddie) is a polyphagous pest that can feed on multiple crop and non-crop hosts.1,2 In the Southeastern U.S., H. zea is multivoltine, completing four or more generations per year.3,4 H. zea feeds on economically important crops including maize (Zea mays L.), cotton (Gossypium hirsutum L.), and soybean (Glycine max L.).3 Genetically engineered maize and cotton expressing Bt Cry endotoxins derived from Bacillus thuringiensis (Bt) Berliner were first commercialized in 1996 to control a suite of lepidopteran pests.5 The primary target pests for Bt maize were Ostrinia nubilalis (Hübner) and Diatraea grandiosella (Dyar) and the target pests for Bt cotton were Chloridea virescens (Fabricius), Pectinophora gossypiella (Saunders), and H. zea.6,7 In maize, H. zea was considered a non-target pest; however, Cry1Ab maize expressed a moderate dose toxin killing between 65–95% of exposed H. zea larvae.7 Since its commercialization, adoption rates for Bt crops have dramatically increased. As of 2020, 82% of the maize and 88% of the cotton planted in the U.S. expressed one or more Bt toxins.8 H. zea resistance to both
Cry1 and Cry2 toxins expressed in maize is widespread across the southern U.S.\textsuperscript{9,10} Furthermore, \textit{H. zea} is not a yield limiting pest of timely-planted maize in the major maize growing regions of the southern U.S.\textsuperscript{11,13} Hence, the greatest concern for \textit{H. zea} as a non-target pest of \textit{Bt} maize is the selection for resistance to \textit{Bt} cotton, where it is a major pest.\textsuperscript{14}

Overwintering \textit{H. zea} typically emerge in May and feed on whorl-stage maize and other non-crop hosts.\textsuperscript{2,4} Generally, the majority of first-generation adult oviposition coincides with silking-stage maize.\textsuperscript{15,16} In North Carolina, maize is an excellent developmental host for the larvae,\textsuperscript{17} and acts as a sink for ovipositing second-generation \textit{H. zea} moths and a source for third-generation \textit{H. zea} moths that disperse to later-season hosts such as cotton and soybean, which they are then more suitable for oviposition.\textsuperscript{4} Late-planted maize can also serve as a source of overwintering \textit{H. zea}.\textsuperscript{18}

Understanding and explaining variation in insect diversity, density, and abundance in agricultural production systems is challenging. The resource concentration hypothesis predicts that herbivores should be more abundant in large patches of host plant\textsuperscript{19} although this hypothesis has weak support.\textsuperscript{20,21} Several empirical studies have found an opposite effect, referred to as the ‘resource diffusion hypothesis’\textsuperscript{22-24} In our study, we sought to determine if there is a relationship between level of damage to maize ears caused by \textit{H. zea} larvae in maize fields and the abundance of maize in the local landscape surrounding those fields. Based on the assumption that populations of \textit{H. zea} would be equally dispersed across available maize in the landscape, our null hypothesis was that populations and associated damage to both \textit{Bt} and non-\textit{Bt} ears within a field would be negatively related to the abundance of maize in the surrounding landscape. To test this hypothesis, we measured \textit{H. zea} larval feeding and development in paired plots of \textit{Bt} maize hybrids expressing Cry1A.105 + Cry2Ab2 and non-\textit{Bt} hybrids across a gradient of maize production intensity in North Carolina and South Carolina. Because ovipositing \textit{H. zea} do not discriminate between \textit{Bt} and non-\textit{Bt} maize,\textsuperscript{25} we did not expect the initial infestation of \textit{Bt} and non-\textit{Bt} maize to differ. However, in the absence of high levels of resistance, we expected \textit{Bt} maize to reduce kernel injury and larval development compared to non-\textit{Bt} maize. In addition to measuring \textit{H. zea} feeding, we used the presence of a larval exit hole as an indicator that a larva completed development in the ear and potentially burrowed in the ground to pupate and later emerge as an adult.\textsuperscript{26}

\section*{2 MATERIALS AND METHODS}
\subsection*{2.1 Maize plots}
From 2017 to 2019, paired \textit{Bt} and non-\textit{Bt} maize plots were planted in maize-growing counties across North Carolina and South Carolina. The \textit{Bt} hybrids included Dekalb 67-72 VT2P and 67-44 VT2P (Genuity VT Double Pro; Bayer CropScience, St. Louis, MO, USA) and both expressed the \textit{Bt} toxins Cry1A.105 + Cry2Ab2. These hybrids were chosen because nearly all the \textit{Bt} maize planted within North Carolina expressed Cry1, a majority expressed Cry2, and very little expressed Vip3Aa20 (7%, 6.5% and 19.3% in 2017, 2018 and 2019, respectively).\textsuperscript{27} The non-\textit{Bt} hybrid was Dekalb 67-70RR in all years (Bayer CropScience). All maize plots ranged from 3–9 m wide and >40 m long. All plots were planted using commercial maize planters at various seeding densities depending on soil type and fertility. Moreover, these plots were nested within commercial fields of corn. Planting dates ranged from late March to early June depending on planting conditions at each site. In 2017, 42-paired (\textit{Bt}/\textit{non-Bt}) plots were planted in North Carolina. In 2018, 29 plots were planted in North Carolina and 22 plots were planted in South Carolina. In 2019, 31 plots were planted in North Carolina and 16 plots were planted in South Carolina. Not all plots had \textit{Bt} and non-\textit{Bt} hybrids planted adjacent to one another, but across all plots the pairs were planted within 50 m of each other.

Plots planted during 2017 and 2019 included all pairs and were planted to 67-70 RR (non-\textit{Bt}) and 67-72 VT2P (Cry1A.105 + Cry2Ab2). In 2018, we were unable to plant 67-72 VT2P in some plots. However, we planted 27 plots to both \textit{Bt} hybrids (67-44 VT2P, 67-72 VT2P) and to 67-70 RR to compare performance of both \textit{Bt} hybrids to each other, as well as 67-70 RR. Seventeen plots were planted exclusively to pairs of 67-70 RR and 67-44 VT2P and seven plots were planted exclusively to pairs of 67-70 RR and 67-72 VT2P. To confirm presence or absence of a \textit{Bt} toxin in each plot, an ELISA strip-test was performed using a composite sample of leaf tissue collected from one leaf on each of three random plants within each plot and following the manufacturer’s instructions (QuickStix Kit for Cry1A/Born Leaf & Seed, Envirologix Inc., Portland, ME, USA). Maize plots at each location were maintained following agronomic recommendations from their respective Cooperative Extension Services. GPS coordinates were recorded at each location using a hand-held device.

\subsection*{2.2 Kernel damage sampling}
To ensure kernel damage was caused by \textit{H. zea}, all plots were sampled for \textit{H. zea} larvae when plants were at the R2-R3 growth stage and when larvae were at least third instars. Kernel damage was measured after \textit{H. zea} had exited the ears, but before feeding by secondary pests (\textit{i.e., Carphophilus lugubris} Murray) had obscured the effects of \textit{H. zea} injury. Between 25–35 mature ears were sampled at random from both \textit{Bt} and non-\textit{Bt} plots at each location, with sample sizes similar to previous studies.\textsuperscript{78-35} Ears were randomly sampled from the middle rows of each plot and at least 5 m inside the plot to reduce the probability of cross-pollinated ears. Previous sampled ears were avoided. The area damaged (cm\textsuperscript{2}) was visually measured on each ear using a gridded 6 cm by 3 cm ruler and the total number of larval exit holes per ear was recorded.

\subsection*{2.3 Landscape analysis}
Landscape composition surrounding each maize plot was determined using remotely sensed data from the USDA National Agricultural Statistics Service-Cropland Data Layer.\textsuperscript{34} The annual production of maize within a 1 km buffer of each sampled field was measured using ArcGIS (Version 10.6.1 ESRI, Redlands, CA, USA). One kilometer was selected based on findings by Graham et al.\textsuperscript{35} that the majority of rubidium-marked \textit{H. zea} emerging in maize fields were captured in traps within 0.8 km radius of the source field. Although both cotton and soybean are important hosts of \textit{H. zea}, they are not an attractive host at the time maize attractiveness to second-generation adults peaks\textsuperscript{17} and, therefore, were not included in our analysis.

\subsection*{2.4 Statistical analysis}
All data analyses were performed in SAS (Version 9.4, SAS Institute, Cary, NC, USA). A Wilcoxon signed-rank test was conducted to determine if there were differences in the total area of damage (cm\textsuperscript{2}) per ear between the two VT2P hybrids, 67-72 and 67-44 using the PROC NPAR1WAY in SAS.\textsuperscript{36} Damaged area per ear did
the GLIMMIX procedure in SAS as a normal distribution using the identity link function using were analyzed using a generalized linear mixed model, modeled the presence of ear damage was ln(x) with percent of sampled ears with at least one larval exit hole as the independent variables. Site location was included as a random effect. To satisfy the normality assumption, ear damage was ln(x + 1) transformed. Means were separated using Tukey's Honestly Significant Difference test with α = 0.05.

To analyze the effects of Bt maize on kernel damage, results were analyzed using a generalized linear mixed model, modeled as a normal distribution using the identity link function using the GLIMMIX procedure in SAS33 where average area of damage per ear (cm²) per plot was coded as the dependent variable and the presence of Bt toxin, year sampled, and their interaction as the independent variables. Site location was included as a random effect. To satisfy the normality assumption, ear damage was ln(x + 1) transformed. Means were separated using Tukey's Honestly Significant Difference test with α = 0.05.

Because our study included multiple sample sites at varying distances from each other, there was the potential for spatial autocorrelation to occur among sites, which would violate the assumption of independence in statistical models. To test for spatial autocorrelation, Moran's test was conducted in R34 using the ape package.37 Using ear damage as the variable of interest, there was significant autocorrelation among sample sites (P = 0.01), indicating that sample locations close to one another had similar amounts of damage. A spherical spatial covariance structure was included to account for spatial autocorrelation among sample sites.

To relate the average area of kernel damage (cm²) and the proportion of larval exit holes per sample to the area of Bt maize within a 1 km radius surrounding each sample site, two generalized linear mixed models, modeled as a normal distribution using the identity link function using the GLIMMIX procedure in SAS36 were constructed. Both models used the same independent variables: proportional area of maize within a 1 km radius of the sample site during the sampling year, presence of Bt toxin, and year sampled. Finally, because the effect of year was significant for kernel damage, we analyzed each year separately to better understand how interannual variation in H. zea activity associated with the intensity of maize production in the surrounding landscape. In contrast, because the effect of year was not significant for the larval exit hole model, we did not analyze each year separately. Site was included as a random intercept term in each model. A spherical spatial covariance structure was included to account for spatial autocorrelation among sample sites. To satisfy the normality assumption, ear damage was ln(x + 1) transformed.

3 RESULTS A total of 4117 Bt and 3504 non-Bt ears of maize across 139 sample locations from 2017 to 2019 were scored for H. zea kernel damage (cm²) and the presence of larval exit holes (Supplemental Table S1). Larval sampling at R3 confirmed that kernel damage was caused exclusively by H. zea. The average kernel damage per ear and percentage of ears with larval exit holes varied by treatment and location during 2017 through 2019. The average area of kernel damage per ear ranged from zero cm² to >37 cm² in both Bt and non-Bt plots (Fig. 1). Similarly, percent of ears with larval exit holes per sample ranged from 0% in both Bt and non-Bt plots to 92% in Bt plots and 100% in non-Bt plots (Fig. 2).

3.1 Bt toxins affect kernel damage The toxin by year interaction was not significant (F = 1.32; df = 2, 161; P = 0.27) indicating that the effect of Bt toxin on the amount of kernel damage did not differ across years. The main effect of Bt toxin on area of kernels damaged was significant (F = 53.56; df = 1, 161; P < 0.01) as was the main effect of year (F = 49.79; df = 2, 161; P < 0.01). On average, Bt maize ears had less kernel damage per ear (5.65 cm² ± 0.58 SEM) than non-Bt maize ears (8.59 cm² ± 0.86). Bt ears had significantly less kernel damage than non-Bt ears in 2017 (F = 14.80; df = 1, 38; P < 0.01), 2018 (F = 29.25; df = 1, 75; P < 0.01), and 2019 (F = 9.10; df = 1, 45; P < 0.01).

3.2 Bt toxin effects on larval development Comparing the percent of larval exit holes per sample in Bt and non-Bt ears, there was not a significant interaction between toxin...
and year ($F = 2.86; \text{df} = 2, 160; P = 0.06$), indicating that the effect of Bt toxins did not differ across years. The percent of ears with larval exit holes per sample was significantly lower in Bt (19% ± 1 SEM) than non-Bt maize (31% ± 2); $F = 66.68; \text{df} = 1, 160; P < 0.01$). The effect of year on the percent of ears with larval exit holes per sample was significant ($F = 16.8; \text{df} = 2, 160; P = 0.82$).

3.3 Effect of the proportional area of surrounding maize composition on kernel damage and larval exit holes

There was a significant negative relationship between kernel damage and the proportional area of maize within 1 km of the sample site ($F = 15.54; \text{df} = 1, 161; P < 0.01$). Because the effect of year sampled was significant ($F = 56.56; \text{df} = 2, 161; P < 0.01$) (Table 1), we analyzed each year individually. In 2017 (Fig. 3(a)) and 2019 (Fig. 3(b)), there was a significant negative relationship between the proportional area of maize during the sample year and the average area of kernel damage per sample ($F = 5.47; \text{df} = 1, 38; P = 0.02$ and $F = 9.32; \text{df} = 1, 45; P = 0.003$, respectively). In 2018, the relationship was not significant ($F = 2.96; \text{df} = 1, 75; P = 0.09$).

Similarly, the percent of ears with larval exit holes per sample significantly decreased as the proportional area of maize in a one km buffer radius during the current year increased ($F = 11.27; \text{df} = 1, 160; P = 0.001$) (Fig. 4; Table 2). Year did not have an effect on the percent of larval exit holes ($F = 0.21; \text{df} = 2, 160; P = 0.81$).

4 DISCUSSION

Because maize is the predominant host for *H. zea* during the period of our study, we analyzed the effects of the current year’s proportional abundance of maize in the landscape surrounding sample fields to understand how the abundance of Bt maize relates to kernel damage and larval exit holes measured in individual maize fields. We found that higher abundance of maize surrounding sample sites during the sampling year was negatively associated with the amount of kernel damage and presence of larval exit holes in maize. Furthermore, the responses (regression slopes) were not significantly different between non-Bt and Bt maize, even though damage and larval exit holes (y-intercepts) were lower in Bt maize. Hence, our null hypothesis that populations and associated damage to both Bt and non-Bt ears within a field would be negatively related to the abundance of maize in the surrounding landscape was accepted. This was a surprising result, given our assumption that maize was a major contributor

**Table 1. Summary of the generalized linear mixed model (final yearly model) results for the average area of kernel damage (cm²) to the proportional area of maize from the current year in a 1 km buffer radius surrounding sample sites by year**

| Year | Response variable | Model parameters | Coefficient estimate | Standard error | t-value | P-value |
|------|-------------------|------------------|----------------------|----------------|---------|---------|
| 2017 | Average damage (cm²) per maize plot (ln(x) + 1) transformed) | Intercept | 3.05 | 0.1546 | 17.61 | <0.0001 |
| 2018 | Average damage (cm²) per maize plot (ln(x) + 1) transformed) | Intercept | 1.65 | 0.1943 | 7.00 | <0.0001 |
| 2019 | Average damage (cm²) per maize plot (ln(x) + 1) transformed) | Intercept | 1.68 | 0.1559 | 9.52 | <0.0001 |

**Figure 2.** Percent larval exit holes in Bt (a) and non-Bt (b) maize at each sample site during 2017–2019. Each dot represents a sample site. Dot size in each figure represents percent larval exit holes per sample.
of *Helicoverpa zea* over population abundance and selective filter for the evolution of *Bt* resistance. Moreover, our results are robust and supported by multiple years, geographical locations, and fine spatial resolution. These results suggest that the presence of resistance to *Bt* Cry toxins manifests as a reduction in efficacy, but not complete loss of control, supporting previous work that resistance to Cry toxins is not uniform across the landscape.\(^9,10\) Importantly, these results illustrate that the current year’s maize abundance is related kernel damage and larval exit holes in maize. This means that observations of variable levels of damage to *Bt* maize across locations cannot be interpreted in terms of resistance levels without first accounting for either *H. zea* infestation levels or the abundance of maize.

Multiple studies have evaluated the performance of *Bt* maize compared to non-*Bt* maize in management of *H. zea* at a limited number of geographical locations.\(^11,29,38\) However, none have evaluated *H. zea* damage in paired *Bt* and non-*Bt* maize plots across a broad geographical region with a large number of trials. Our results demonstrate that *Bt* maize expressing Cry1A.105 + Cry2Ab2, compared to non-*Bt* maize, reduced kernel damage from *H. zea*, indicating that resistance levels were not uniform across North Carolina and South Carolina. However, *Bt* maize reduced kernel damage by only 35% ± 0.02 SEM (min. 0; max. 100%) compared to previous studies (2010–2012) in *Bt* maize expressing the same toxins (Cry1A.105 + Cry2Ab2) which...
reported reductions of 90 to 95%. Note that Yang et al. studied maize hybrids that expressed Cry1A.105 + Cry2Ab2 + Cry1F. However, Cry1F has only sub-lethal effects on H. zea and does not reduce rates of feeding. Our study differed from these previous studies in several important ways. First, we evaluated kernel damage across a broad geography. In addition, our exit hole analysis demonstrated that almost 20% of H. zea potentially completed development in Bt ears and successfully exited the ear as compared to 32% in non-Bt. However, not all H. zea completing development in an ear create an observable exit hole in the husk, as some may exit the ear through the silk channel. Therefore, our larval exit hole data may underestimate the number of larvae completing development in the ear. Because H. zea can successfully develop on maize expressing Cry1A.105 + Cry2Ab2, pupate, eclose, and produce viable offspring, this finding suggests that Bt maize is contributing Bt-resistant H. zea that may later infest Bt cotton.

Our data correspond with previous findings that regional suppression of lepidopteran pests can be attributed to widespread planting of Bt crops in with Bt crops act as a trap crop, which has been demonstrated for O. nubilalis, Helicoverpa armigera (Hüber), and H. zea. However, our results were collected from multiple locations investigating the effect of maize abundance in the landscape on a local level (1 km radius). The slopes of the regression of kernel damage and pupal exit holes to maize abundance in the landscape were remarkably similar between Bt and non-Bt maize and among years. This indicates that, although plantings of hybrids expressing Vip3Aa20 varied across years (7%, 6.5% and 19.3% hybrids planted in 2017, 2018 and 2019, respectively, expressed Vip3Aa20), it did not influence our findings. In addition, the difference in response between non-Bt ears and Bt ears was not different among years (i.e., the difference in slopes). Consequently, other hypotheses may explain our results. For example, pest dilution is a density-dependent effect in which pest densities (number of pests per plant) decrease with an increasing abundance of a host. Crowding occurs as pest densities increase in tandem with decreasing host abundance because the population is distributed over greater or lesser abundance of suitable hosts. In this study, we did not measure the overall abundance of H. zea in each landscape. Accepting the assumption of pest dilution, and if we assume populations are equally distributed across a landscape, when more maize is present in the landscape, instabations of H. zea should be lower in individual maize fields. This is because the presence of more maize produces more ovipositional sites for a given H. zea population. In contrast, when less maize is present in the landscape instabations of H. zea should be higher in individual maize fields. Several studies have observed these effects. For example, Ricci et al. observed lower trap catches of Cydia pomonella L. as the proportional area of pome fruit orchards increased in a 500 m buffer. Similarly, Zaller et al. observed decreased damage from Cetorrhynchus assimilis and Dasineura brassicae in winter oilseed rape (Brassica napus L.) fields as the proportional area of winter oilseed rape fields increased within a 2 km buffer. In our study, we observed a negative relationship between the proportional area of maize in the local landscape and the amount of kernel damage and the proportion of ears with a larval exit hole aligning with the results of the previous pest dilution studies.

In conclusion, we demonstrated that Bt maize expressing Cry1A.105 + Cry2Ab2 reduced kernel damage from H. zea relative to non-Bt maize; however, the suppression level was lower than reported in previous studies, suggesting higher levels of resistance may negate the suppression effect. Furthermore, our larval exit hole analysis illustrated that Bt maize is likely contributing Bt resistant H. zea to the landscape. Finally, we demonstrated that maize abundance in the local landscape influenced kernel damage and the number of larval exit holes in maize. Importantly, our study demonstrated that although levels of kernel damage to Bt maize are greater than previously reported, Bt maize (toxins) continue to reduce ear damage and suppress the overall population of H. zea at a localized level.

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DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

SUPPORTING INFORMATION

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

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