

# Competitive release and outbreaks of non-target pests associated with transgenic *Bt* cotton

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**Abstract.** The adoption of transgenic *Bt* cotton has, in some cases, led to environmental and economic benefits through reduced insecticide use. However, the distribution of these benefits and associated risks among cotton growers and cotton-growing regions has been uneven due in part to outbreaks of non-target or secondary pests, thereby requiring the continued use of synthetic insecticides. In the southeastern USA, *Bt* cotton adoption has resulted in increased abundance of and damage from stink bug pests, *Euschistus servus* and *Nezara viridula* (Heteroptera: Pentatomidae). While the impact of increased stink bug abundance has been well-documented, the causes have remained unclear. We hypothesize that release from competition with *Bt*-susceptible target pests may drive stink bug outbreaks in *Bt* cotton. We first examined the evidence for competitive release of stink bugs through meta-analysis of previous studies. We then experimentally tested if herbivory by *Bt*-susceptible *Helicoverpa zea* increases stink bug leaving rates and deters oviposition on non-*Bt* cotton. Consistent with previous studies, we found differences in leaving rates only for *E. servus*, but we found that both species strongly avoided ovipositing on *H. zea*-damaged plants. Considering all available evidence, competitive release of stink bug populations in *Bt* cotton likely contributes to outbreaks, though the relative importance of competitive release remains an open question. Ecological risk assessments of *Bt* crops and other transgenic insecticidal crops would benefit from greater understanding of the ecological mechanisms underlying non-target pest outbreaks and greater attention to indirect ecological effects more broadly.

**Key words:** *Bt* cotton; *Gossypium hirsutum*; induced resistance; non-target effects; Pentatomidae; pest outbreak; transgenic insecticidal crops.

## INTRODUCTION

Insect-resistant transgenic *Bt* cotton, *Gossypium hirsutum* L. (Malvaceae), has provided effective management and even regional suppression of serious target lepidopteran pests of cotton (Naranjo et al. 2008, NRC 2010). At the same time, some secondary pests that are not susceptible to *Bt* toxins have become more serious threats to cotton production in some regions (NRC 2010, Catarino et al. 2015). Mirid and lygaeid plant bugs have become more serious pests associated with *Bt* cotton adoption in India and Pakistan (Patil and Rajanikantha 2005, Rohini et al. 2009, Patil et al. 2010). Likewise, adoption of *Bt* cotton in China has driven species of mirid plant bugs, namely *Apolygus lucorum* Meyer-Dür, *Adelphocoris suturalis* (Jakovlev), *Adelphocoris fasciaticollis* (Reuter), and *Adelphocoris lineolatus* (Goeze), from

secondary to major pest status (Bergé and Ricroch 2010, Lu et al. 2010). Importantly, these non-target pest outbreaks have contributed to continued high use of insecticides by some adopters of *Bt* cotton, eroding the expected environmental and economic advantages of the technology (Pemsl et al. 2005, Yang et al. 2005, Wang et al. 2008, Arshad et al. 2009).

Likewise, in the southeastern USA, outbreaks of an assemblage of stink bug pests, primarily *Euschistus servus* Say, but also *Nezara viridula* L. and *Chinavia hilaris* (Say) (Heteroptera: Pentatomidae), are associated with high adoption rates of *Bt* cotton (Turnipseed et al. 1995, Zeilinger et al. 2011). While *Bt* cotton, particularly Bollgard II and other gene-stacked lines, has resulted in an overall reduction in insecticide use in the southeastern USA, insecticide use targeting stink bugs has risen substantially since the commercial release of *Bt* cotton (Greene et al. 2006, Herbert et al. 2009, Zeilinger et al. 2011).

To explain non-target pest outbreaks associated with *Bt* cotton, reduced broad-spectrum insecticide use has been marshalled as the dominant hypothesis in the literature (Greene et al. 2001, Lu et al. 2010, reviewed in Catarino

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et al. 2015). If secondary pest populations are managed by high insecticide rates typical of non-*Bt* cotton systems (Naranjo et al. 2008), then reduced insecticide rates in *Bt* cotton may indeed lead to secondary pest outbreaks (Lu et al. 2010). However, insecticide release may be insufficient to fully explain new outbreaks. Rather, additional mechanisms may be necessary for a full understanding.

Historically, ecological work on non-target or secondary pest outbreaks associated with synthetic insecticides has focused on the role of natural enemy release (reviewed in Risch 1987 and Hardin et al. 1995). Nonetheless, additional mechanisms have been shown to be important in some cases, including hormoligosis (Dittrich et al. 1974), changes in plant quality (Chelliah and Heinrichs 1980), and competitive release (Root and Skelsey 1969). More recently, mechanisms other than natural enemy release have become more widely accepted, particularly changes in plant quality and competitive release (Szczeplaniec et al. 2011, 2013, Cordeiro et al. 2014, Guedes and Cutler 2014). This change could be due to contemporary shifts toward more selective insecticidal classes (e.g., neonicotinoids; Boyd and Boethel 1998, Goulson 2013) and/or the growing recognition among ecologists that bottom-up and competitive interactions can strongly influence herbivore populations (Kaplan and Denno 2007, Karban 2011).

Some authors have suggested that reductions in *Bt*-susceptible caterpillar populations in *Bt* fields could lead to competitive release of non-target pest populations (Sujii et al. 2006, Naranjo et al. 2008, Catarino et al. 2015). Competitive release in *Bt* crops would require strong competitive interactions among pest species in non-*Bt* fields. Because of the apparently ample resources for pests in monoculture agroecosystems, such competition would most likely be characterized as interference competition mediated through induced plant responses (Hagenbucher et al. 2013b), although alternatives are possible (Dorhout and Rice 2010).

We hypothesize that the severity of stink bug outbreaks in cotton in the southeastern USA are at least partially determined by the population densities of *Bt*-susceptible competitors, namely the heliothine species *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae). If so, competitive release may play an important role in stink bug outbreaks in *Bt* cotton. Herbivory by *H. zea* larvae on non-*Bt* cotton plants reduces nymphal growth rates of *E. servus* by 36% at within-plant scales and deters feeding by *E. servus* nymphs at the whole-plant scale (Zeilinger et al. 2011, 2015). Moreover, the species-specific and multi-scale nature of these interactions indicate that they are mediated by induced resistance in the shared host plant (Zeilinger et al. 2015).

We aim to further test the competitive release hypothesis. First, we conducted a meta-analysis of previous studies to assess the overall strength and consistency of competitive effects. We next experimentally tested stink bug leaving rates in field settings and oviposition preference at the whole-plant scale between *H. zea*-damaged and

undamaged non-*Bt* cotton plants. Specifically, we predict that *H. zea* herbivory will (1) increase leaving rates and deter oviposition by stink bugs and (2) that competitive effects will be stronger for *E. servus* than for *N. viridula*, as measured in both meta-analysis and new experiments.

Our studies on leaving rates in the field and oviposition preference provide further insight into heliothine-stink bug interactions. Inter-plant movement of herbivores, including leaving rates, can be a strong indicator of demographic effects from inter-plant variation in resistance (either constitutive or induced; Bernays 1999, Underwood 1999). At the same time, oviposition preference can be an important driver of herbivore population dynamics (Ohgushi 1995, Heard and Remer 2008), in part because oviposition preference is often more sensitive than other life history traits to variation in plant quality (Gripenberg et al. 2010) and the presence of competitors (Kaplan and Denno 2007). By spatially separating the stink bug from the heliothine, the experiments are designed to test for plant-mediated competition, which has been shown to be a strong and frequent form of interspecific competition among herbivorous insects (Kaplan and Denno 2007). We show strong and consistent competitive effects from *H. zea* on *E. servus* performance, behavior, and oviposition preference, and that these competitive effects are plant-mediated rather than resource or direct interference competition.

## MATERIALS AND METHODS

### *Meta-analysis of previous studies*

To assess competitive effects from heliothine larvae (*H. zea* and *H. virescens*) on stink bug performance (*E. servus* and *N. viridula*), we compiled previously published results on their interactions into a meta-analysis. We calculated the effect size as Hedges's *d* from competition experiments described in Zeilinger et al. (2011, 2015). Each species combination consisted of  $n = 4$  effect sizes, except the *N. viridula* and *H. virescens* combination in which  $n = 3$ .

### *Leaving rate experiment*

We conducted the leaving rate experiment at the Bellflower Experimental Farm, USDA-ARS, Tifton, Georgia, USA. Non-*Bt* glyphosate-tolerant transgenic cotton (DP147, RoundUp Ready Flex) was planted in two 300-m<sup>2</sup> plots. Planting and cultivation practices and insect colony rearing were as described in Zeilinger et al. (2011). Using 10-12 week-old plants (and excluding plants growing <3 m from the field edge), we randomly assigned each plant to two treatments: (1) undamaged control plants or (2) plants damaged by *H. zea*. To obtain caterpillar damage, we caged two third-instar larvae separately on two mid-level main-stem leaves for 72 h. Plants were excluded from the experiment if any caterpillar herbivory was evident prior to the treatments; plants with the damage treatment were excluded if no herbivory occurred after 36 h. A total sample size of 46

and 56 damaged plants were obtained for *E. servus* and *N. viridula* trials, respectively.

Three days after heliothine damage began, we removed the larvae and the cages and placed a single adult stink bug female of either species, 5–7 days old since molting, within the bracts of the first position (top of plant) boll. Trials with *E. servus* were conducted in 2011; trials with *N. viridula* were conducted in 2012. The trials were started at 21:00 as stink bugs are most active at night (Shearer and Jones 1996). An infrared head lamp was used to observe stink bug location on the plant. The location of each stink bug was recorded as being on the original boll (boll 1), on a different part of the plant (e.g., meristem, leaf one, flower 1, boll 2, etc.), or off the plant at 10 min, 1 h, 12 h, and 24 h from the start for *E. servus*, and up to 36 h for *N. viridula*. While experimental stink bugs were not marked, naturally occurring stink bugs were unlikely to confound our observations as the experimental plants were checked thoroughly for the absence of stink bugs prior to the start of trials. Additionally, the plots were adjacent to fruiting soybean plots, which are highly preferred over cotton for colonization, leading to low populations in cotton plots at the time of our study (Olson et al. 2011).

#### *Oviposition preference*

Greenhouse-grown DP147 cotton plants were grown at the USDA-ARS station, Tifton, Georgia, USA, as described in Zeilinger et al. (2015). To set up the experiment, we paired two non-*Bt* cotton plants according to the size of the uppermost fully mature main stem leaf and randomly assigned a treatment (heliothine damaged, undamaged) to one of the pair. Similar to the leaving rate experiment, heliothine damage was obtained by confining one third instar *H. zea* on each of two mid-level leaves with a 23.76-cm<sup>2</sup> clip cage for 72 h.

For both stink bug species, pairs in copula were removed from the colony cages and placed in smaller screened cages (5 × 12 cm). When mating ceased, we removed the male, and the female was left in the cage with food (a cotton boll) and water for an additional 3 d before the preference trial, which is the majority of the pre-oviposition period and served to decrease the time to oviposition in the experiment (Harris and Todd 1980).

Three days after *H. zea* larvae began feeding on the cotton plants, the uppermost cotton leaf on the damaged, and undamaged plants were placed in opposite ends of a wire and wood cage (42 × 30 × 4 cm). One chilled, immobile gravid female stink bug was placed in the neutral space between the leaves along with a moistened cotton wick and two freshly picked, undamaged cotton bolls, one by each leaf, which were food sources for the bugs. The cage consisted of two oval-shaped metal screens framed with wood (4 × 2 cm) and clamped together. Grooves (0.5 cm h on each of the two framed screens) were cut into the wood at the point where the leaves of each plant were inserted into the cage to prevent petiole damage. Both cotton leaves remained connected to the plants and neither

leaf was damaged, thereby testing stink bug responses to systemically induced plant resistance. The cage was checked daily for an egg mass and plants were discarded when oviposition was observed. If oviposition had not occurred by the second day, an additional third instar *H. zea* was caged on another leaf of the plant to maintain the induced plant defense. The trial was stopped after 4 d if oviposition had not occurred. This occurred three times for *N. viridula* and 41 times for *E. servus*. To obtain enough replicates, trials with *E. servus* were conducted over a 2-yr period. Total sample sizes were 17 and 20 for *E. servus* and *N. viridula* oviposition preference trials, respectively.

The location of the egg mass (undamaged leaf, damaged leaf, cage wall) was recorded for each trial, and for trials with *E. servus*, we also recorded the number of eggs in the egg mass and the area of each leaf in the arena. To obtain leaf area, we took an RGB digital image of each leaf, selected all pixels representing the leaf, based on color segmentation of the green component of the image. The minimum threshold pixel value was 10; the maximum pixel value varied between 125 and 160, based on histogram values of each green component. We then divided the number of pixels representing the leaf by the number of pixels in a 2.5 cm line in the digital image to obtain the leaf area. MATLAB 9.1 (MathWorks, Natick, MA) was used for digital image processing.

#### *Statistical analysis*

For the meta-analysis, we analyzed the compiled competition effect sizes using a mixed-effects linear model (Koricheva et al. 2013). Stink bug species and heliothine species were fixed effects and study was included as a random effect. There were two stink bug species and two lepidopteran species, resulting in four combinations of the fixed effects.

For the leaving rate experiment, we analyzed leaving rates from whole plants. Differences in leaving rates between herbivory treatments were analyzed with Cox parametric survival models. The assumption of constant leaving rates was tested by model selection between exponential (constant hazard rate) and Weibull hazard functions (Machin et al. 2006, Crawley 2007).

For *E. servus*, we investigated the independence of oviposition choice on year and leaf area with a three-way log-linear contingency table analysis. Leaf area was coerced into a binomial variable by coding each leaf in a pair as the larger or smaller leaf of the pair. Differences in leaf area (as a continuous variable) between damaged and undamaged plants were also analyzed with a Wilcoxon paired-sample test. Oviposition choice was then analyzed using a one-way log-linear model with William's correction for each stink bug species (Sokal and Rohlf 1981). We tested for differences in the number of eggs per egg mass between undamaged and damaged plants with a one-way ANOVA.

All analyses were conducted in R 3.2.0 (R Core Team 2015). We used the lmer package for the mixed-effects

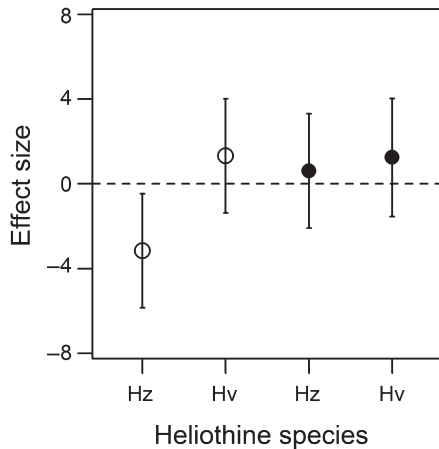


FIG. 1. Results of a meta-analysis of previous competition experiments between stink bugs (*E. servus* and *N. viridula*; open and filled circles, respectively) and heliothine larvae (*H. zea* and *H. virescens*; Hz and Hv, respectively). Estimates are from a mixed-effects linear model and 95% confidence intervals were calculated using the likelihood profile method. A negative effect size indicates a competitive interaction. Points indicate mean effect size and error bars indicate  $\pm$  95% confidence intervals.

meta-analysis (Bates et al. 2014). For the log-linear analysis, we used the `g.test()` function from Hurd (2001) and verified the results by hand. Survival analyses were conducted using the `survival` package (Therneau 2015).

## RESULTS

### Meta-analysis

Combining effect sizes from previous studies indicated that there were significant species-specific effects from heliothine damage on stink bug responses ( $n = 3\text{--}4$ ,

$F_{4,11} = 4.46$ ,  $P = 0.022$ ). *Euschistus servus* experienced strong and statistically significant competitive effects from *H. zea* damage on shared cotton plants (Fig. 1). Effect sizes for the other heliothine–stink bug combinations were not significantly different from 0.

### Leaving rates

Akaike's information criterion (AIC) values for the exponential and Weibull leaving rate models for *N. viridula* were equivalent (AIC<sub>exponential</sub> = 385.00, AIC<sub>Weibull</sub> = 385.70), so the simpler (exponential) model was chosen. This indicates that the leaving rate was constant during the experiment. For *E. servus* on the other hand, the Weibull regression model was a better fit (AIC<sub>Weibull</sub> = 175.06, AIC<sub>exponential</sub> = 233.78), meaning that leaving rates were not constant. The estimated scale parameter was  $2.17 \pm 1.12$  (SE), indicating increasing leaving rates over time. Leaving rates for *N. viridula* did not differ between herbivory treatments ( $n = 56$ ,  $Z = 0.762$ ,  $P = 0.446$ ), whereas *E. servus* leaving rates were significantly higher from *H. zea*-damaged plants than from undamaged plants ( $n = 46$ ,  $Z = 2.99$ ,  $P = 0.00282$ ; Fig. 2).

### Oviposition preference

Females of both species oviposited approximately 2 d after the beginning of the trials, or 5 d after mating. *Helicoverpa zea* larvae often consumed the entire leaf area within the leaf cage (23.76 cm<sup>2</sup>) by the end of the trial. Oviposition choice, year, and leaf size were all independent of each other (choice vs. leaf size,  $df = 1$ , Dev = 0.262,  $P = 0.609$ ; choice vs. year,  $df = 1$ , Dev = 0.384,  $P = 0.535$ ; year vs. leaf size,  $df = 1$ , Dev = 0.130,  $P = 0.718$ ; choice vs. year vs. leaf size,  $df = 1$ , Dev = 2.53,  $P = 0.112$ ). The areas of leaves presented to stink bugs were not

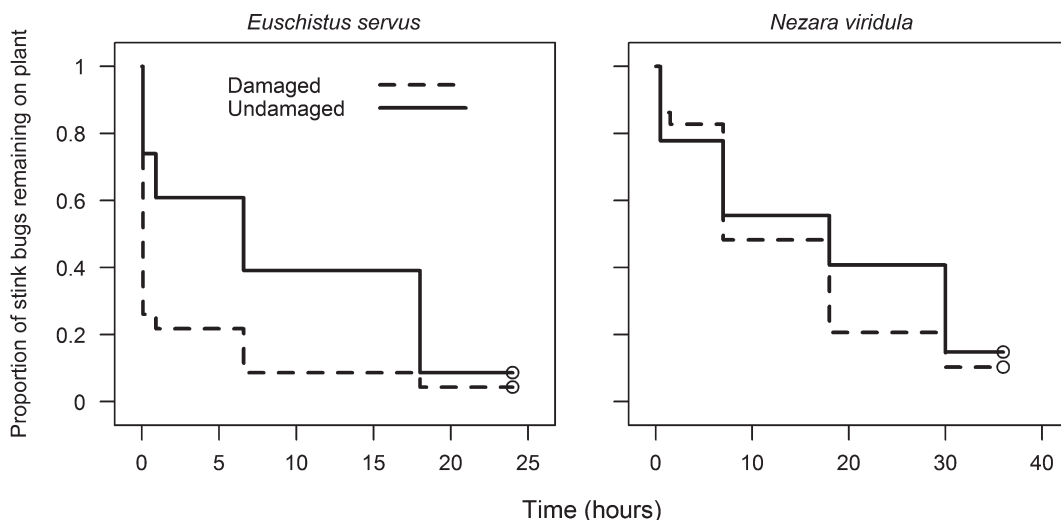


FIG. 2. Kaplan–Meier survival plots showing the proportion of stink bugs (*E. servus* or *N. viridula*) remaining on cotton plants damaged by *H. zea* or left undamaged (dashed and solid lines, respectively). *Euschistus servus* left damaged plants significantly faster than undamaged plants according to a Cox parametric survival model ( $n = 46$ ,  $Z = 2.99$ ,  $P = 0.00282$ ), but leaving rates for *N. viridula* were not significantly different ( $n = 56$ ,  $Z = 0.762$ ,  $P = 0.446$ ).

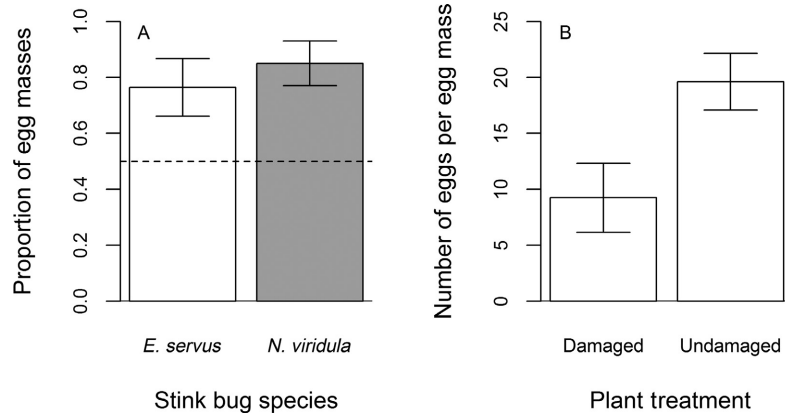


FIG. 3. (A) Proportions of oviposition by *E. servus* and *N. viridula* females on undamaged cotton plants were significantly greater than oviposition on *H. zea* damaged plants according to one-way G tests (*N. viridula*:  $n = 20$ ,  $df = 1$ ,  $G = 10.5$ ,  $P = 0.001$ ; *E. servus*:  $n = 17$ ,  $df = 1$ ,  $G = 4.9$ ,  $P = 0.027$ ). (B) Mean number of eggs per *E. servus* egg mass found on leaves of undamaged plants was significantly greater than number of eggs found on damaged plants according to a one-way ANOVA ( $n_{\text{damaged}} = 4$ ,  $n_{\text{undamaged}} = 13$ ,  $F_{1,15} = 6.3$ ,  $P = 0.024$ ). Error bars are  $\pm$  SE.

significantly different between damaged and undamaged plants ( $n = 13$ ,  $T_s = 34$ ,  $P = 0.4548$ ).

We did not find more than one egg mass per female during any trial, and only one egg mass was found on a cage wall; all others were found on the underside of a cotton leaf. For both stink bug species, significantly more egg masses were found on the undamaged than the damaged plant (Fig. 3; *N. viridula*:  $n = 20$ ,  $df = 1$ ,  $G = 10.5$ ,  $P = 0.001$ ; *E. servus*:  $n = 17$ ,  $df = 1$ ,  $G = 4.9$ ,  $P = 0.027$ ). *Euschistus servus* and *N. viridula* females oviposited  $76.5 \pm 10.3\%$  ( $\pm$  SE) and  $85.0 \pm 8.0\%$  of the time on undamaged plants, respectively. For *E. servus*, egg masses on undamaged plants contained significantly more eggs than egg masses on damaged plants (Fig. 3;  $n_{\text{damaged}} = 4$ ,  $n_{\text{undamaged}} = 13$ ,  $F_{1,15} = 6.3$ ,  $P = 0.024$ ). Egg masses found on damaged plants had  $9.25 \pm 3.09$  eggs per egg mass (mean  $\pm$  SE), whereas undamaged plants had  $19.62 \pm 2.54$  eggs per egg mass.

## DISCUSSION

The widespread adoption of *Bt* cotton has resulted in many ecological and agronomic changes, including dramatic shifts in pest community assemblages (NRC 2010, Lu et al. 2010, Catarino et al. 2015). Increasing dominance of certain non-target pests may be driven by reduced competition with target pests. Competitive release of non-target species, as a mechanism for new outbreaks, is possible only where competition exists between target and non-target species in non-*Bt* cotton environments.

We found strong repellent effects of *H. zea* herbivory on *E. servus* in the field; *E. servus* left damaged plants significantly faster than undamaged plants, even though damage occurred only on two leaves. *Nezara viridula*, on the other hand, showed no differences in leaving rates. We also found a strong oviposition preference of *E. servus* and *N. viridula* females for cotton plants that

were not damaged by *H. zea* larvae. Both species oviposited on undamaged plants in more than 75% of trials, and *E. servus* females oviposited twice as many eggs per egg mass on undamaged than damaged plants. These results corroborate our meta-analysis results indicating strong and consistent competitive effects from *H. zea* herbivory on *E. servus*.

The interactions between *H. zea* and *E. servus* are best explained through induced plant responses. Our experimental designs excluded possible effects from resource and direct interference modes of competition. Herbivory by *H. zea* larvae on cotton plants induces a complex and diverse set of systemic changes in plant quality (reviewed in Hagenbucher et al. 2013a), though the particular traits driving *E. servus* response remain unclear (Zeilinger et al. 2015).

The competitive effects from *H. zea* on *E. servus* described here and in previous studies could produce population-level effects. Naturally occurring heavy infestations of *H. zea* in southeastern insecticide-free non-*Bt* fields can cause nearly complete yield loss, with every plant being damaged (Greene et al. 2011), while 40–50% of bolls are damaged under moderate *H. zea* infestations (Benedict et al. 1996). Furthermore, with a low economic threshold for stink bugs of one bug per two row-meters (Greene et al. 2001), small changes in stink bug densities could have large impacts on cotton yield. Given this, and that our *H. zea* herbivory treatments were localized and for a short time, our estimates of the magnitude of competitive effects are likely conservative.

In addition to reducing the quality of damaged plants, ecological theory predicts *H. zea* damage could further suppress *E. servus* populations by increasing the heterogeneity of plant quality within a field. Heterogeneity in host plant quality, due to interspecific plant diversity, intraspecific genotypic diversity, or induced resistance, often suppresses herbivore populations through associational

TABLE 1. Previous studies show significant competitive effects between *Bt*-target pests and *Bt*-resistant non-target pests.

<i>Bt</i> -target pest	Non-target pest	Crop species	Competitive mechanism	Reference
<i>Heliothis virescens</i> (F.)	<i>Aphis gossypii</i> (Glover)	cotton	plant-mediated (terpenoids)	Hagenbucher et al. (2013b)
<i>Spodoptera littoralis</i> (Boisd.)	<i>Rhopalosiphum maidis</i> (Fitch)	maize	plant-mediated (VOCs)	Bernasconi et al. (1998)
<i>Ostrinia nubilalis</i> (Hübner)	<i>Sriacosta albicosta</i> (Smith)	maize	resource competition	Dorhout and Rice (2010)
<i>Spodoptera litura</i> (F.)	<i>Nilparvata lugens</i> Stål	rice	plant-mediated (VOCs)	Xu et al. (2002)

resistance (Andow 1991, Underwood 1999b, Cronin and Abrahamson 2001, Zakir et al. 2013, but see Rodriguez-Saona and Thaler 2005). Heterogeneity in plant quality effectively increases travel costs for ovipositing females by increasing distance between high quality hosts (Heard and Remer 2008) and can reduce tenure time on both high and low quality hosts (Bernays 1999). Similarly, *H. zea* herbivory increased *E. servus* leaving rates from both damaged and nearby undamaged plants (Zeilinger et al. 2015). The prevailing evidence makes clear that under heavy to moderate *H. zea* infestations, non-*Bt* cotton fields constitute patches of poorer average host quality and greater host heterogeneity for *E. servus* relative to *Bt* cotton fields.

Evidence is accumulating for the role of competitive release in determining non-target pest outbreaks associated with *Bt* crops. While still few in number, studies examining interactions among *Bt*-susceptible target and *Bt*-resistant non-target pest species on their shared crop hosts show a consistent pattern of competitive effects on non-target species (Table 1). These studies also suggest that plant-mediated interactions may be common, as has been found for interspecific interactions among herbivorous insects more broadly (Kaplan and Denno 2007).

While competitive release and insecticide release are both important drivers of stink bug outbreaks in *Bt* cotton, other processes should also be considered. Stink bugs are not controlled by natural enemies in southeastern cotton fields (Jones et al. 1996, Olson and Ruberson 2012), so the absence of effective natural enemies probably also contributes to the stink bug outbreaks. Finally, it is possible that the landscape structure has favored the outbreaks of these polyphagous stinkbugs; in the southeastern USA, maize and soybean are common alternative hosts, on which stink bugs can reproduce and build up populations before cotton becomes a suitable host (Toews and Shurley 2009).

The quantification of indirect adverse effects in ecological risk assessments has been a persistent challenge (USEPA 1998) and have been generally ignored in most risk assessment frameworks for GE crops (Andow et al. 2013). Nonetheless, the competitive release of non-target pests highlights the importance of indirect effects. Understanding the full range of mechanisms driving non-target pest outbreaks will be critical for rigorous risk assessments and will provide regulators, policy-makers, and growers with improved predictions of

changes in insecticide use and yield with adoption of future transgenic crops.

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#### LITERATURE CITED

- Andow, D. A. 1991. Vegetational diversity and arthropod population response. *Annual Review of Entomology* 36: 561–586.
- Andow, D. A., G. L. Lovei, S. Arpaia, L. Wilson, E. M. Fontes, A. Hilbeck, D. M. F. Capalbo, K. Prescott, C. Omoto, and A. R. Zeilinger. 2013. An ecologically-based method for selecting ecological indicators for assessing risks to biological diversity from genetically-engineered plants. *Journal of Biosafety* 22:141–156.
- Arshad, M., A. Suhail, M. D. Gogi, M. Yaseen, M. Asghar, M. Tayyib, H. Karar, F. Hafeez, and U. N. Ullah. 2009. Farmers' perceptions of insect pests and pest management practices in *Bt* cotton in the Punjab, Pakistan. *International Journal of Pest Management* 55:1–10.
- Bates, D., M. Maechler, B. Bolker and S. Walker. 2015. lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-9, URL: <https://CRAN.R-project.org/package=lme4>.
- Benedict, J. H., E. S. Sachs, D. W. Altman, W. R. Deaton, R. J. Kohel, D. R. Ring, and S. A. Berberich. 1996. Field performance of cottons expressing transgenic CryIA insecticidal proteins for resistance to *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctuidae). *Journal of Economic Entomology* 89:230–238.
- Bergé, J. B., and A. E. Ricroch. 2010. Emergence of minor pests becoming major pests in GE cotton in China: What are the reasons? What are the alternatives practices to this change of status? *GM Crops* 1:214–219.
- Bernasconi, M. L., T. C. Turlings, L. Ambrosetti, P. Bassetti, and S. Dorn. 1998. Herbivore-induced emissions of maize volatiles repel the corn leaf aphid *Rhopalosiphum maidis*. *Entomologia Experimentalis et Applicata* 87:133–142.

- Bernays, E. A. 1999. When host choice is a problem for a generalist herbivore: experiments with the whitefly, *Bemisia tabaci*. *Ecological Entomology* 24:260–267.
- Boyd, M. L., and D. J. Boethel. 1998. Susceptibility of predaceous Hemipteran species to selected insecticides on soybean in Louisiana. *Journal of Economic Entomology* 91:401–409.
- Catarino, R., G. Ceddia, F. J. Areal, and J. Park. 2015. The impact of secondary pests on *Bacillus thuringiensis* (*Bt*) crops. *Plant Biotechnology Journal* 13:601–612.
- Chelliah, S., and E. A. Heinrichs. 1980. Factors affecting insecticide-induced resurgence of the brown planthopper, *Nilaparvata lugens* on rice. *Environmental Entomology* 9:773–777.
- Cordeiro, E. M. G., A. S. Corrêa, and R. N. C. Guedes. 2014. Insecticide-mediated shift in ecological dominance between two competing species of grain beetles. *PLoS ONE* 9:e100990.
- Crawley, M. J. 2007. *The R book*. Wiley, New York, New York, USA.
- Cronin, J. T., and W. G. Abrahamson. 2001. Goldenrod stem galler preference and performance: effects of multiple herbivores and plant genotypes. *Oecologia* 127:87–96.
- Dittrich, V., P. Streibert, and P. A. Bathe. 1974. An old case reopened: mite stimulation by insecticide residues. *Environmental Entomology* 3:534–540.
- Dorhout, D. L., and M. E. Rice. 2010. Intraguild competition and enhanced survival of Western bean cutworm (Lepidoptera: Noctuidae) on transgenic Cry1Ab (MON810) *Bacillus thuringiensis* corn. *Journal of Economic Entomology* 103:54–62.
- Goulson, D. 2013. An overview of the environmental risks posed by neonicotinoid insecticides. *Journal of Applied Ecology* 50:977–987.
- Greene, J. K., S. G. Turnipseed, M. J. Sullivan, and O. L. May. 2001. Treatment thresholds for stink bugs (Hemiptera : Pentatomidae) in cotton. *Journal of Economic Entomology* 94:403–409.
- Greene, J. K., C. S. Bundy, P. M. Roberts, and B. R. Leonard. 2006. Identification and management of common boll feeding bugs in cotton. Clemson University, Blackville, South Carolina, USA.
- Greene, J. K., D. M. Robinson, K. M. Carter and G. M. Devinney. 2011. Performance of new and existing *Bt* cotton technologies when inundated with heavy/natural populations of bollworm in SC-2010. Pages 993–999 *Proceedings of the Beltwide Cotton Conference*. Cotton, Atlanta, Georgia, USA.
- Gripenberg, S., P. J. Mayhew, M. Parnell, and T. Roslin. 2010. A meta-analysis of preference–performance relationships in phytophagous insects. *Ecology Letters* 13:383–393.
- Guedes, R. N. C., and G. C. Cutler. 2014. Insecticide-induced hormesis and arthropod pest management. *Pest Management Science* 70:690–697.
- Hagenbucher, S., D. M. Olson, J. R. Ruberson, F. L. Wäckers, and J. Romeis. 2013a. Resistance mechanisms against arthropod herbivores in cotton and their interactions with natural enemies. *Critical Reviews in Plant Sciences* 32:458–482.
- Hagenbucher, S., F. L. Wäckers, F. E. Wettstein, D. M. Olson, J. R. Ruberson, and J. Romeis. 2013b. Pest trade-offs in technology: reduced damage by caterpillars in *Bt* cotton benefits aphids. *Proceedings of the Royal Society B* 280:20130042.
- Hardin, M. R., B. Benrey, M. Coll, W. O. Lamp, G. K. Roderick, and P. Barbosa. 1995. Arthropod pest resurgence: an overview of potential mechanisms. *Crop Protection* 14:3–18.
- Harris, V. E., and J. W. Todd. 1980. Temporal and numerical patterns of reproductive-behavior in the southern green stink bug, *Nezara viridula* (Hemiptera, Pentatomidae). *Entomologia Experimentalis et Applicata* 27:105–116.
- Heard, S. B., and L. C. Remer. 2008. Travel costs, oviposition behaviour and the dynamics of insect–plant systems. *Theoretical Ecology* 1:179–188.
- Herbert, A., E. Blinka, J. Bachelier, J. W. Van Duyn, J. K. Greene, M. D. Toews, P. Roberts, and R. H. Smith. 2009. Managing stink bugs in cotton: research in the southeast region. Virginia Cooperative Extension, Blacksburg, Virginia, USA.
- Hurd, P. 2001. Log-likelihood test of independence and goodness of fit.
- Jones, W. A., B. M. Shepard, and M. J. Sullivan. 1996. Incidence of parasitism of Pentatomid (Heteroptera) pests of soybean in South Carolina with a review of studies in other states. *Journal of Agricultural Entomology* 13:243–263.
- Kaplan, I., and R. F. Denno. 2007. Interspecific interactions in phytophagous insects revisited: a quantitative assessment of competition theory. *Ecology Letters* 10:977–994.
- Karban, R. 2011. The ecology and evolution of induced resistance against herbivores. *Functional Ecology* 25:339–347.
- Koricheva, J., J. Gurevitch, and K. Mengersen. 2013. *Handbook of meta-analysis in ecology and evolution*. Princeton University Press, Princeton, New Jersey, USA.
- Lu, Y., K. Wu, Y. Jiang, B. Xia, P. Li, H. Feng, K. A. Wyckhuys, and Y. Guo. 2010. Mirid bug outbreaks in multiple crops correlated with wide-scale adoption of *Bt* cotton in China. *Science* 328:1151–1154.
- Machin, D., Y. B. Cheung, and M. K. Parmar. 2006. *Survival analysis: a practical approach*, Second edition. John Wiley & Sons, West Sussex, UK.
- Naranjo, S. E., J. R. Ruberson, H. C. Sharma, L. Wilson, and K. Wu. 2008. The present and future role of insect-resistant genetically modified cotton in IPM. Pages 159–193 *in* J. Romeis, A. M. Shelton and G. G. Kennedy, editors. *Integration of insect-resistant genetically modified crops within IPM programs*. Springer Science + Business Media, New York, New York, USA.
- NRC [National Research Council]. 2010. *Impact of genetically engineered crops on farm sustainability in the United States*. National Academies Press, Washington, D.C., USA.
- Ogushi, T. 1995. Adaptive behavior produces stability in herbivorous lady beetle populations. Pages 303–319 *in* N. Cappuccino and P. W. Price, editors. *Population dynamics: new approaches and synthesis*. Academic Press, San Diego, California, USA.
- Olson, D. M., and J. R. Ruberson. 2012. Crop-specific mortality of southern green stink bug eggs in *Bt*- and non-*Bt* cotton, soybean and peanut. *Biocontrol Science and Technology* 22:1417–1428.
- Olson, D. M., J. R. Ruberson, A. R. Zeilinger, and D. A. Andow. 2011. Colonization preference of *Euschistus servus* and *Nezara viridula* in transgenic cotton varieties, peanut and soybean. *Entomologia Experimentalis et Applicata* 139:161–169.
- Patil, B. V., and R. Rajanikantha. 2005. Dusky cotton bug: a future threat to *Bt* cotton cultivation. *Insect Environment* 11:77–79.
- Patil, S. B., S. S. Udikeri, N. B. Vandal, S. A. Modagi, R. B. Hirekurubar, and G. S. Guruprasad. 2010. Population dynamics of *Creontiades biseratense* (Distant) (Miridae: Hemiptera) on *Bt* cotton in Dharwad district. *Karnataka Journal of Agricultural Sciences* 23:157–158.
- Pemsl, D., H. Waibel, and A. P. Gutierrez. 2005. Why do some *Bt*-cotton farmers in China continue to use high levels of pesticides? *International Journal of Agricultural Sustainability* 3:44–56.
- R Core Team. 2015. *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Risch, S. J. 1987. Agricultural ecology and insect outbreaks. Pages 217–238 *in* P. Barbosa and J. C. Schultz, editors. *Insect outbreaks*. Academic Press, San Diego, California, USA.

- Rodriguez-Saona, C., and J. S. Thaler. 2005. Herbivore-induced responses and patch heterogeneity affect abundance of arthropods on plants. *Ecological Entomology* 30:156–163.
- Rohini, R. S., C. P. Mallapur, and S. S. Udikeri. 2009. Incidence of mirid bug, *Creontiades biseratense* (Distant) on *Bt* cotton in Karnataka. *Karnataka Journal of Agricultural Sciences* 22:680–681.
- Root, R. B., and J. J. Skelsey. 1969. Biotic factors involved in crucifer aphid outbreaks following insecticide application. *Journal of Economic Entomology* 62:223–233.
- Shearer, P. W., and V. P. Jones. 1996. Diel feeding pattern of adult female southern green stink bug (Hemiptera: Pentatomidae). *Environmental Entomology* 25:599–602.
- Sokal, R. R., and F. J. Rohlf. 1981. *Biometry*. W.H. Freeman, New York, New York, USA.
- Sujii, E. R., G. L. Lovei, M. Setamou, P. Silvie, M. G. Fernandes, G. S. J. Dubois and R. P. Almeida. 2006. Non-target and biodiversity impacts on non-target herbivorous pests. Pages 133–154 in A. Hilbeck, D. A. Andow, and E. M. G. Fontes, editors. *Environmental risk assessment of genetically modified organisms, Volume 2: methodologies for assessing Bt cotton in Brazil*. CABI, Oxfordshire, UK.
- Szczepaniec, A., S. F. Creary, K. L. Laskowski, J. P. Nyrop, and M. J. Raupp. 2011. Neonicotinoid insecticide imidacloprid causes outbreaks of spider mites on elm trees in urban landscapes. *PLoS ONE* 6:e20018.
- Szczepaniec, A., M. J. Raupp, R. D. Parker, D. Kerns, and M. D. Eubanks. 2013. Neonicotinoid insecticides alter induced defenses and increase susceptibility to spider mites in distantly related crop plants. *PLoS ONE* 8:e62620.
- Therneau, T. 2015. A package for survival analysis in S.
- Toews, M. D., and W. D. Shurley. 2009. Crop juxtaposition affects cotton fiber quality in Georgia farmscapes. *Journal of Economic Entomology* 102:1515–1522.
- Turnipseed, S. G., M. J. Sullivan, J. E. Mann and M. E. Roof. 1995. Secondary pests in transgenic *Bt* cotton in South Carolina. Pages 768–769. Cotton, San Antonio, Texas, USA.
- Underwood, N. 1999a. The influence of plant and herbivore characteristics on the interaction between induced resistance and herbivore population dynamics. *American Naturalist* 153:282–294.
- [USEPA] U.S. Environmental Protection Agency. 1998. Guidelines for ecological risk assessment. U.S. Environmental Protection Agency, Washington, D.C., USA.
- Wang, S., D. R. Just, and P. Pinstrip-Andersen. 2008. *Bt* cotton and secondary pests. *International Journal of Biotechnology* 10:113–121.
- Xu, T., Q. Zhou, Q. Xia, W. Zhang, G. Zhang, and D. Gu. 2002. Effects of herbivore-induced rice volatiles on the host selection behavior of brown planthopper, *Nilaparvata lugens*. *Chinese Science Bulletin*. 47:1355–1360.
- Yang, P., M. Iles, S. Yan, and F. Jolliffe. 2005. Farmers' knowledge, perceptions and practices in transgenic *Bt* cotton in small producer systems in Northern China. *Crop Protection* 24:229–239.
- Zakir, A., M. M. Sadek, M. Bengtsson, B. S. Hansson, P. Witzgall, and P. Anderson. 2013. Herbivore-induced plant volatiles provide associational resistance against an ovipositing herbivore. *Journal of Ecology* 101:410–417.
- Zeilinger, A. R., D. M. Olson, and D. A. Andow. 2011. Competition between stink bug and heliothine caterpillar pests on cotton at within-plant spatial scales. *Entomologia Experimentalis et Applicata* 141:59–70.
- Zeilinger, A. R., D. M. Olson, D. MacLean, N. Mori, R. Nakata, and D. A. Andow. 2015. Behavioral and chemical mechanisms of plant-mediated deterrence and attraction among frugivorous insects. *Ecological Entomology* 40:532–542.

## DATA AVAILABILITY

Data associated with this paper have been deposited in Dryad: <http://dx.doi.org/10.5061/dryad.51591>