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Determining Yield Effects of Simulated Stand Loss in Field Corn (*Zea mays*)

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Abstract

The corn borer complex, consisting of *Diatraea grandiosella* (Dyar), *Ostrinia nubilalis* (Hübner), and *D. saccharalis* (F.), poses a risk to field corn that is not protected through the use of foliar-applied insecticides or *Bacillus thuringiensis* (Bt) proteins incorporated into the plant's genetics. In the southern U.S., corn borers have been adequately controlled through the widespread planting of Bt corn hybrids. Refuge systems have been implemented to prevent the selection of Bt-resistant populations in target species. Historically, structured refuge compliance among corn producers has been low, leading to the commercialization of seed blended refugia in areas of the U.S. where cotton is not grown. It could be assumed that if seed blended refugia were approved for use in corn in the southern U.S., producers would not manage corn borers leading to the possible loss of refuge plants. To determine how a complete loss of refuge plants would affect yield, insect-related plant population loss was simulated at various levels ranging from 0% to 50% in 10% increments. Plant population loss was simulated at both the V5 and V10 growth stages. In low yielding environments, every one percent loss in plant population resulted in a 26.6 Kg Ha^{-1} reduction in corn yield. Subsequently, in high yielding environments, every one percent loss in plant population resulted in a 78.86 Kg Ha^{-1} reduction in corn yield. Results suggest that in a situation where a seed blended refuge was implemented into the mid-southern U.S., significant yield losses could be observed if refuge plants are left unprotected in the presence of high corn borer populations.

Keywords: stand loss, yield, field corn, borer complex, refuge, *Bacillus thuringiensis*

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Introduction

The borer complex of corn, *Zea mays* (L.), consists of the European corn borer, *Ostrinia nubilalis* (Hübner); the southwestern corn borer, *Diatraea grandiosella* (Dyar); and the sugarcane borer, *D. saccharalis* (F.). *O. nubilalis* and *D. saccharalis* tend to be uncommon pests in the mid-southern U.S., and *D. grandiosella* populations are more commonly observed in the region (Baldwin et al. 2006). Both *O. nubilalis* and *D. saccharalis* have become more common across the southernmost Gulf States which may be due to reduced tillage practices and increasing corn hectares across the region (Castro et al. 2004, Huang et al. 2006) *D. grandiosella* is periodic in occurrence and damage may be greater than realized due to damage being hidden in the stalk (Davis et al. 1933). *O. nubilalis* is considered the most damaging insect pest of corn in the U.S. and Canada with losses exceeding one billion dollars each year (Ostlie et al. 1997). The feeding behaviors and damage to corn among species in the borer complex are relatively similar. Adults lay eggs on leaves of corn and grain sorghum, *Sorghum bicolor* (L.) Moench. Larvae hatch and feed on foliage for a short time before boring into the stalk and feeding within the vascular tissue which leads to disruption of the movement of water and nutrients (Culy 2000, Baldwin et al. 2005). This feeding can result in stunting, plant deformation, deadheart, and sometimes plant death (Culy 2000). Tissue injury caused by this complex can also lead to stalk lodging and ear drop contributing to yield reductions (Edwards et al. 1992). Later planted corn typically experiences more *D. grandiosella* damage than early planted corn suggesting that earlier planted corn will mature before damaging infestation levels can occur (Starks et al. 1982, Baldwin et al. 2005).

Corn hybrids that express insecticidal proteins from the soil bacterium, *Bacillus thuringiensis* (Bt) var. *kurstaki* (Bt), have provided excellent control of the corn borer complex and other lepidopteran pests (Ostrý et al. 2015). To prevent the selection of resistant alleles in target insects, a high-dose refuge strategy was implemented as a method of insecticide resistance management (IRM) (US EPA 1998). Several assumptions must be met for structured refuges to effectively delay resistance in target species. The assumptions are that the Bt must kill >99.9% of the wild-type individuals, the resistance allele is rare, resistance is mostly recessive, and random mating occurs between moths emerging from the Bt crop and refuge crop (Onstad and Knolhoff 2014). Initially, refuge deployment involved planting non-Bt corn hybrids in structured blocks separate from the Bt crop or as strips of non-Bt hybrids within the Bt field (Onstad et al. 2017). Recently, seed blended refugia were approved in some regions of the U.S. for dual-gene Bt corn hybrids. This consists of a certain number of refuge seed mixed with Bt seed.

Structured refuge compliance has been low among producers leading to lower Bt susceptible insect populations and increased selection for Bt resistance alleles in *H. zea* (Reisig 2017). A benefit of planting a seed blended corn refuge is that it maximizes adult mixing in a field setting as refuge plants would be distributed randomly across Bt fields. Blended seed refuge also places the burden of refuge deployment on the seed distributor instead of the producer (Carroll et al. 2012, Onstad et al. 2014). Low refuge compliance is especially an issue in cotton-growing areas because both corn and cotton express the same or similar Bt proteins and *Helicoverpa zea* (Boddie) feeds on both crops in succession (Von Kanel et al. 2016). For this reason, corn seed blended refugia are being considered as an option in cotton-growing areas to slow the development of resistance.

The potential introduction of seed blends as a refuge option in the mid-southern U.S., would likely change the way producers protect field corn. Structured refugia can be easily treated with foliar insecticides based on recommended action thresholds because they are typically planted in blocks or strips. However, because seed blended refugia plants would be randomly dispersed across a field of majority Bt protected plants, it is likely that producers would not try to protect these refuge plants because the whole field would have to be sprayed. Untreated refuge plants are left vulnerable to attack from corn borers as well as other pests. In a worst-case scenario infestation of corn borers where extreme damage or even death to refuge plants occurs, it is possible to have negative effects such as reduced overall crop yield and decreased production of susceptible insects from the refuge. This experiment examines potential yield loss in a seed blended field corn refuge when deployed at various non-Bt percentages. Additionally, loss of refuge plants can decrease the overall size and effectiveness of the refuge regarding Bt susceptible adult production.

Materials and Methods

A field study was conducted from 2017 to 2019 to determine how various percentages of simulated insect damage could affect yield in field corn. During 2017, this study was conducted at the R. R. Foil Plant Science Research Center in Starkville, Mississippi and the Delta Research and Extension Center in Stoneville, Mississippi. In 2018, this study was conducted at the R. R. Foil Plant Science Research Center in Starkville, Mississippi. During 2019 the same study was conducted in two separate fields at two different planting dates at the R. R. Foil Plant Science Research Center in Starkville, Mississippi. Planting dates for each trial in Starkville were 3 May in 2017, 12 April and 9 May in 2018, 29 May and 16 June in 2019. The planting date for the trial in Stoneville was 9 May in 2017. Field experiments were arranged as a randomized complete block with a 2 x 6 factorial arrangement of treatments and four replications. This study was repeated for a total of five site years. The factors included plant population loss timing and percent stand loss. To determine if corn can compensate for stand loss in early and mid-vegetative growth stages, stand loss timings were imposed at the V5 and V10 growth stages. Percent plant population loss treatments were 0%, 10%, 20%, 30%, 40% and 50%. Stand loss occurred by mixing the appropriate percentage of non-Roundup Ready corn seed (Conv. ZS7987) with glyphosate [N-(phosphomethyl) glycine] (Roundup®, Monsanto Company, St. Louis, MO) resistant corn seed (DEKALB® DKC67-72, Monsanto Company, St. Louis, MO). The two cultivars were mixed thoroughly for random in-plot trait distribution. This occurred for every planting row within each plot. Two packages of equal amounts of blended seed (one package per row) were prepared for each plot. Seed were planted using an Almaco plot research specific cone-planter (Almaco, Nevada, IA). Corn was planted at the Starkville, MS location in two-row plots at a rate of 79,040 seeds per hectare on 96.5-cm row beds at a depth of 3.81-cm below soil level. At the Stoneville, MS location, corn was planted in two-row plots on 101.6-cm row beds at a depth of 3.81-cm below the soil level. Plots were 12.2-m in length at both locations. Corn seed was treated with clothianidin at a rate of 0.5 mg ai/seed to protect plants from early-season underground insect pests. To determine initial plant populations, stand counts were recorded at the V3 growth stage before termination events by counting every live plant in each plot.

Glyphosate was applied to designated plots at the V5 and V10 growth stages at a rate of 1.54 kg ai ha^{-1} to terminate glyphosate susceptible plants and to achieve the desired plant population loss percentage. Plant population counts were recorded ten days after termination events to confirm termination success. Plots were maintained weed-free across all locations through hand weeding and the application of pre-emergence and post-emergence herbicides. Fertilizer applications were based on soil test recommendations across locations. Furrow irrigation was utilized in experiments that were conducted in Stoneville, MS, but not in Starkville, MS. At maturity, the entire plot was harvested, yields and percent moisture were recorded. Before analysis, corn grain yields were standardized to 15% moisture for all plots. Trials were harvested using a research scale combine with a weigh system and moisture meter.

In the initial analysis, yield data were analyzed using a mixed model analysis of variance (SAS Institute 2019) to determine how stand loss events affect corn yields. Test (site year), plant loss timing, plant loss percentage, and all interactions were considered fixed effects in the model. Replication, replication nested in test, and replication by plant loss timing nested in test were considered random effects in the model. In this analysis, there was a significant test by percent plant loss interaction (Table 1). The effect of test was then analyzed using LSMEANS and mean yields among tests were separated based on Tukey's HSD (α = 0.05). Starkville 2017 and Starkville 2018 tests were grouped into high yielding environments while the location in Stoneville 2017 and the two Starkville locations in 2019 were grouped into low yielding environments (Fig. 1). Corn yields in each environment were analyzed with regression analysis (SAS Institute 2019). For each plant loss timing within an environment, plant population loss percentage was the independent variable and corn grain yield was the dependent variable. Analysis of covariance was used to test the slopes of the regression equations between the two plant loss timings within each environment. The slopes of the regression equations were not different between plant loss at V5 and V10 as indicated by a non-significant plant loss timing by plant loss percentage interaction in the low yielding (F = 1.29; df = 1, 140; *P* = 0.26) and high yielding (F = 0.15; df = 1, 92; *P* = 0.70) environments. As a result, data within each environment were combined across plant loss timings for corn yields. Analysis of covariance was used for the final analysis to test the slopes of the regression equations across the low yielding and high yielding environments. For all regression analyses, both linear and quadratic terms were tested for each model.

Results

There were no significant differences in plant populations among treatments prior to glyphosate applications being made (F = 0.58; df = 5, 200.9; *P* = 0.71). Based on plant population counts, the method used to blend the glyphosate-resistant and conventional seed was an effective means of simulating stand loss in a field setting (Fig. 2). The plant stand termination strategy had a significant effect on plant populations at the V5 (F = 41.01; df = 5, 80.27; *P* <0.01) and V10 (F = 37.33; df = 5, 90; *P* <0.01) growth stages. After the V5 terminations, plant populations in the different percent plant loss treatments were different from each other, except that plant populations in the 40% and 50% plant loss treatments were similar to each other (Fig. 2). The 10, 20, 30, 40, and 50% plant loss treatments resulted in plant populations that were 91, 82, 71, 60, and 52% of the zero plant loss treatment, respectively. After the V10 terminationsplant populations in the different percent plant loss treatments were different from each other except the plant populations in the 20% and 30% and the 30% and 40% plant loss treatments were similar to each other (Fig. 2). The 10, 20, 30, 40, and 50% plant loss treatments resulted in plant populations that were 91, 78, 70, 64, and 52% of the zero plant loss treatment, respectively.

Table 1. Results of the analysis of variance evaluating yield effects of stand loss in field corn across 5 site years in Mississippi in 2017, 2018, and 2019.

Effect	*F*	df	*P*
Test	16.75	12	<0.01
Timing	0.15	15	0.70
Timing*Test	0.98	15	0.45
Percent Loss	11.29	190	0.01
Percent Loss*Test	2.82	190	0.03
Percent Loss*Timing	1.62	190	0.20
Timing*Percent Loss*Test	0.61	190	0.66

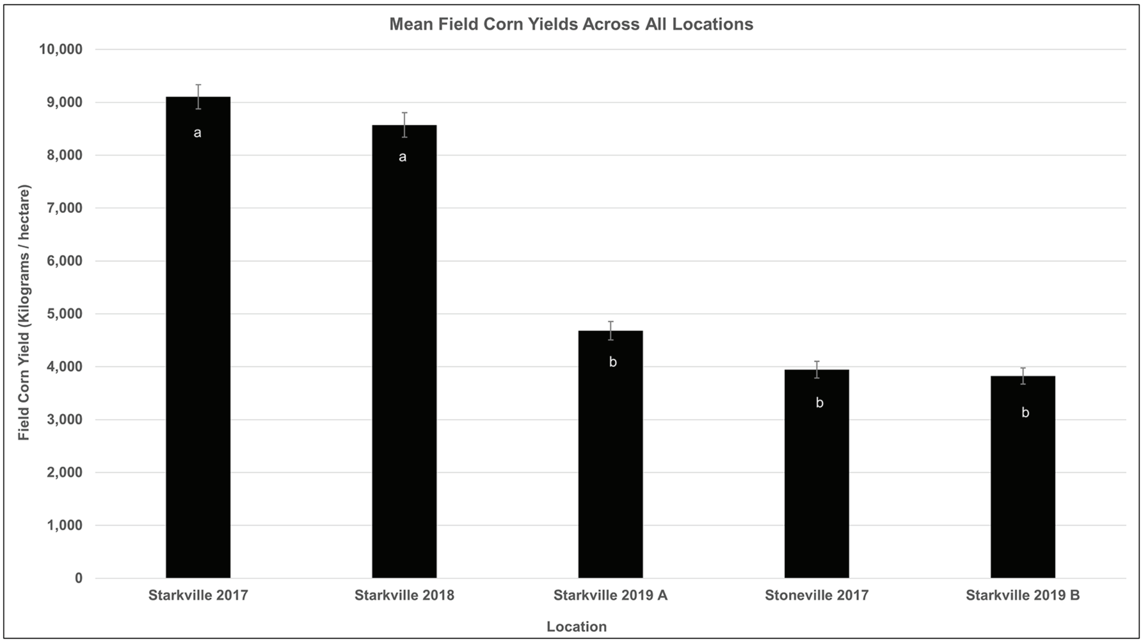

Figure 1. Means ± SEM yields across all locations from experiments evaluating stand loss in field corn. LS means with the same letter are not significantly different (Tukey-groupings of LS means, α = 0.05).

The percent plant loss by yield environment interaction was significant (F = 43.98; df = 1, 236; *P* <0.01) suggesting that the response of corn to plant loss was different between the low yielding and high yielding environments (Fig. 3). In the low yielding environment, there was a linear relationship between plant loss percentage and corn grain yield when averaged across plant loss timings (F = 9.98; df = 3, 140; *P* <0.01). For every one percent loss in plant population, there was a 26.6 Kg Ha^{-1} reduction in corn yield (Fig. 3). Subsequently, in high yielding environments, there was a significant linear relationship between plant loss and corn grain yield when averaged across plant loss timings (F = 75.74; df = 3, 92; *P* <0.01). For every one percent loss in plant population, there was a 78.86 Kg Ha^{-1} reduction in corn yield (Fig. 3).

Discussion

The introduction of Bt incorporated crops has provided near-complete control of *O. nubilalis* and *D. grandiosella* while reducing insecticide applications (Ostlie et al. 1997). Surveys conducted by Rice and Ostlie (2013) concluded that producers typically did not manage *O. nubilalis* because yield losses were not always obvious, they were unwilling to scout for the pest, history suggested no previous pest problems, and failure to recognize the cause of yield loss, among many other reasons. The corn borer complex can be a serious pest in both sweet corn and field corn due to stalk and shank tunneling, causing plant lodging or ear drop (Capinera 2000, Bessin 2012). Unlike *H. zea*, corn borers can be gregarious feeders leading to multiple individuals per plant resulting in greater yield loss potential (Chiang et al. 1960). Previous research has shown that *D. grandiosella* could cause 8% to 100% yield losses in dent stage field corn (Walton and Bieberdorf 1948). However, small plants are more susceptible to corn borer injury, so early season cultural practices are encouraged to promote large healthy plants before corn borer

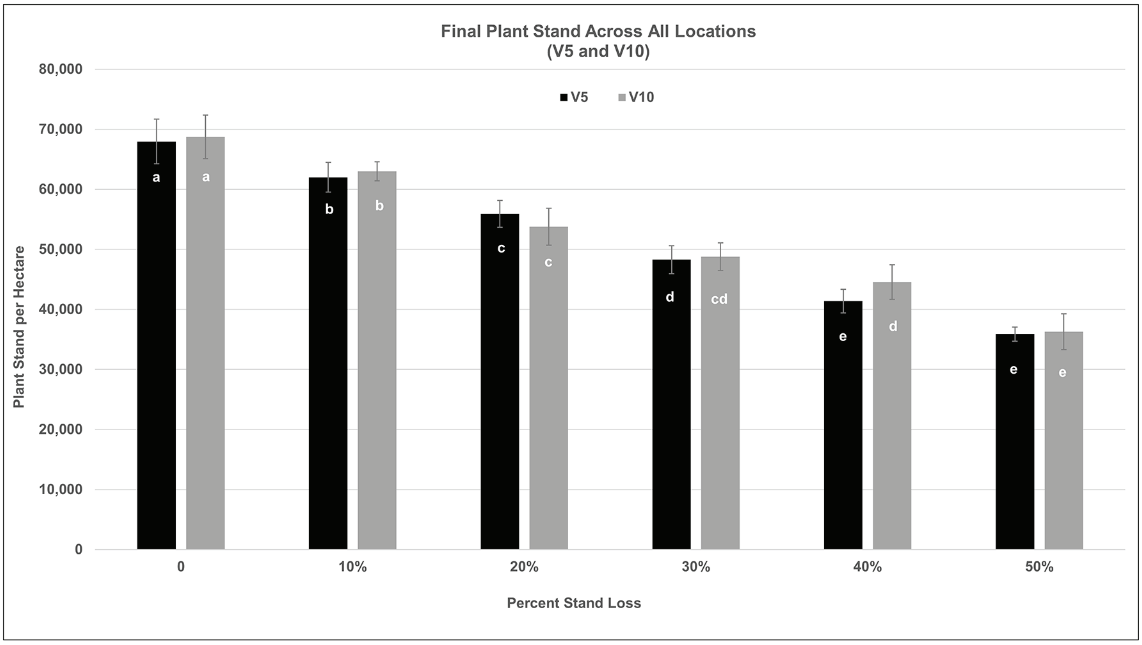

Figure 2. Means ± SEM plant population from stand loss treatments at the V5 and V10 growth stage of corn plants, averaged across experiments conducted in Starkville and Stoneville, Mississippi from 2017 to 2019. LS means with the same letter are not significantly different (Tukey-groupings of LS means, α = 0.05).

establishment (Arbuthnot et al. 1958). Moving to a seed blended refuge could potentially put producers at risk of yield losses from the corn borer complex. Seed blended refugia would essentially ensure that refuge compliance is met, however, it could lead to unprotected corn plants and the possibility of significant yield losses if large enough populations become established. In the current study, yield losses from severe simulated corn borer injury resulted in 26.6 Kg Ha^{-1} and 78.9 Kg Ha^{-1} yield losses for every one percent loss in plant populations in low yield and high yield environments, respectively.

Another factor to consider is the effect that stand loss has on yield regarding corn compensation. Even though corn boring species are not always a cause of significant yield loss, it is likely that insect damage resulting in lowered plant population and, thus, decreased plant uniformity could have negative effects on yield. Research has documented that grain yield increases when plant spacing uniformity increases due to increased photosynthesis and decreases plant stress during yield determining growth stages (Andrade et al. 2002). Additionally, improved genetics and breeding techniques have led to newer hybrids that produce higher yields when planted at higher plant densities in comparison to older hybrids (Duvick 2005). Another factor that could affect yield in high plant densities is maximizing photosynthetically active radiation (PAR) interception, which can be achieved through reaching total ground cover rather quickly because there is a strong relationship between increased PAR interception and grain yield (Andrade et al. 2002). To ensure total ground coverage happens as quickly as possible, stand loss occurrence should be prevented. Plant crowding leads to greater leaf area indices as well as reduced leaf width and increased leaf angle allowing for higher PAR penetration further into the corn canopy (Bernhard and Below 2020).

As technology improves, crop producers have more options regarding farming equipment and implements. This new technology benefits

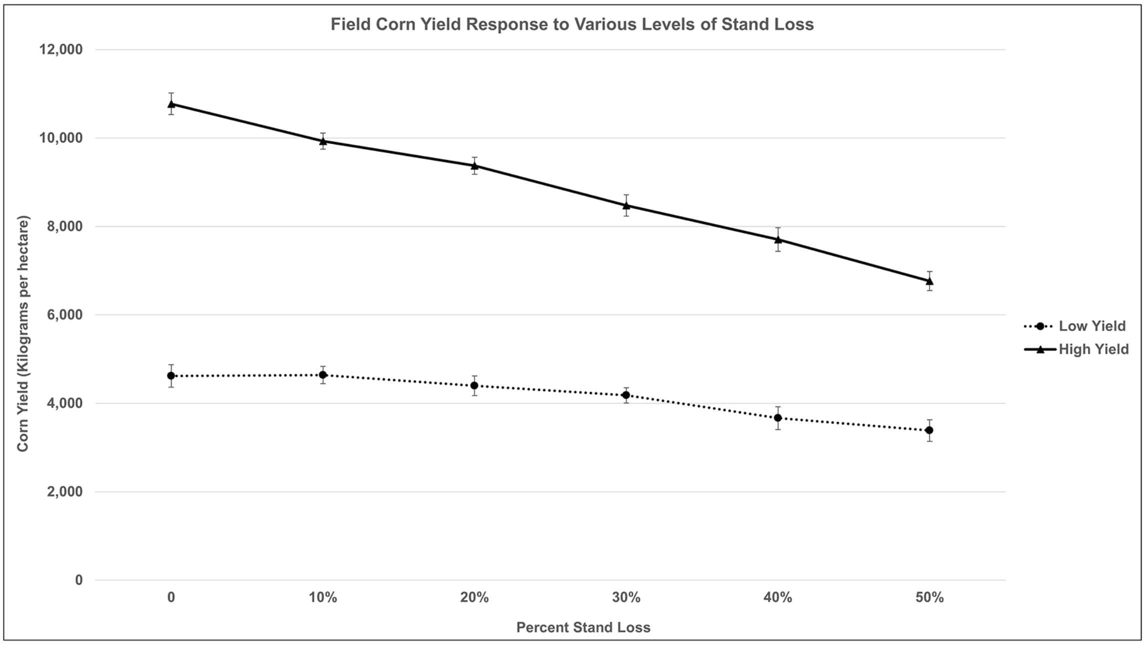

Figure 3. Impacts of varying levels of stand loss on corn yield in high and low yielding scenarios in environments in Mississippi from 2016 to 2019.

(▲) $y = -78.86x + 10809$; $P < 0.01$
(●) $y = -26.57x + 4814$; $P < 0.01$

growers in the Southeastern U.S. due to being able to consistently plant seed at ideal plant populations and seeding depth in highly variable soil types (Virk et al. 2019). Adequate soil moisture within the row bed most often affects germination and emergence and is the key to uniform seedling establishment and maximizing yield potential (Carter et al. 1989, Virk et al. 2019). The introduction of precision planters allows producers to reduce seeding rates while achieving ideal plant populations, whereas, previously, planting rates were adjusted to be slightly higher to compensate for at-planting variability. With improved planting methods and uniform stand establishment leading to reduced seeding rates, plant stand must be protected to maximize yield potential. In a scenario of field corn planted with a precision planter, it is likely that even minuscule amounts of stand loss, whether insect-related or not, could lead to yield reductions. In a seed blended refuge scenario, this effect could be amplified because a certain percentage (≥5%) of seed planted would be non-Bt and would remain unprotected from lepidopteran insect feeding unless scouted and protected through the use of foliar insecticides. Additionally, it is more important than ever to plant field corn seed that is treated with an insecticide seed treatment to protect from the soil insect complex and various seedling pests. Field corn seedlings that have not been treated with a systemic insecticide seed treatment can be significantly reduced in the presence of soil-dwelling pest pressure (North et al. 2018).

Although the current study investigated a worst-case scenario for corn borer injury, the data suggest that some risk of yield loss may be recognized from planting a seed blended refuge. Scouting and the implementation of a comprehensive trapping program would be required to monitor populations, however, this may be unlikely due to the intensive nature of scouting for corn borer infestations. With the introduction of precision planting equipment that allows for the decrease of seed input, protecting field corn stand is now much more important. Corn boring insect infestations could likely be detrimental in seed blended refuge incorporated corn planted using precision planters. Future research looking at actual infestations of corn borer spp. in seed blended refugia incorporated fields would be beneficial to supplement this study.

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