Research Article

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Larval western bean cutworm feeding damage encourages the development of Gibberella ear rot on field corn

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Abstract

BACKGROUND: A 2 year study was conducted to determine whether western bean cutworm (*Striacosta albicosta* Smith) (WBC) larval feeding damage increases severity of the fungal disease Gibberella ear rot [*Fusarium graminearum* (Schwein.) Petch] in field corn (*Zea mays* L.). The effect of a quinone-outside inhibiting fungicide, pyraclostrobin, on Gibberella ear rot severity and mycotoxin production, both with and without WBC pressure, was also evaluated. The impact of each variable was assessed individually and in combination to determine the effect of each upon ear disease severity.

RESULTS: There was a positive correlation between the presence of WBC larvae in field corn and Gibberella ear rot severity under inoculated conditions in the 2 years of the experiment. An application of pyraclostrobin did not impact Gibberella ear rot development when applied at corn growth stage R1 (silks first emerging).

CONCLUSION: Feeding damage from WBC larvae significantly increases the development of *F. graminearum* in field corn. We conclude that an effective integrated management strategy for Gibberella ear rot should target the insect pest first, in an effort to limit disease severity and subsequent mycotoxin production by *F. graminearum* in kernels.

Keywords: *Striacosta albicosta*; western bean cutworm; *Fusarium graminearum*; fungicide

1 INTRODUCTION

The United States is the top-ranked nation for corn (commonly referred to as maize) production and export in the world. During the 2013 growing season, 13.8 billion bushels (over 349 million metric tons) of corn were harvested from 39 million hectares.[1] Grain prices have been volatile in recent years, and growers now focus on in-season preventive pest management practices to increase yields, as opposed to scouting and monitoring methods that were traditionally part of the integrated pest management (IPM) approach.[2] These management tactics typically target both potential insect and disease threats. For example, in the early 2000s, the western bean cutworm [*Striacosta albicosta* (Smith)] (WBC) emerged as a potential threat to corn production in the upper Midwest.[3] WBC has been an established pest of dry beans and field and sweet corn in Nebraska, Idaho and Colorado, and has successfully expanded its range eastward into additional corn-producing states. In 2006, captures of male moths in traps baited with WBC female sex pheromone confirmed the presence of the moth in Indiana. It has since been found in several other Midwestern states and Ontario, Canada.[3]

Larvae of WBC are univoltine, feeding on leaves, tassels, silks and ears of corn. Eggs are deposited upon corn in the whorl stage, usually on the upper surface of the newest leaves. After hatching, WBC larvae consume the empty egg cases, then migrate to the whorl where they feed on pollen.[4] Larvae later move to the developing ear, entering via the silk channel to begin feeding on kernels at the ear tip.[5] Various types of ear damage can be sustained during larval feeding, ranging from surface scraping of kernels to complete consumption of individual kernels. Larvae of WBC also enter and leave ears by chewing through the husk, causing direct economic loss due to feeding damage. One recent study estimated that WBC reduces yield by 37.2 bushels ha^{-1} at a rate of 1 larva plant^{-1}.[6]

Insect feeding damage to corn ears is also a concern owing to the possibility of pathogen entry and spread. There are several damaging ear rot fungi that may co-occur with WBC, and insect feeding or mechanical damage on corn ears could serve as entry points for pathogens. One of these, *Fusarium graminearum*, is a fungal pathogen that infects ears at silking, especially during cool and wet weather conditions. *F. graminearum* causes the disease Gibberella ear rot, and is characterized by a pinkish-white mat of mycelium that covers the ear and eventually leads to reduced grain quality and yield loss.[7,8] The fungus primarily gains entry

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to the ear through the silk channel;[9] disease severity is highest when infection occurs on young or newly emerged silks, but it may also enter ears through wounds.[10–12] *F. graminearum* also produces toxic compounds, called mycotoxins, as a byproduct of the infection process. The most common mycotoxins associated with *F. graminearum* infection are deoxynivalenol (DON), also known as vomitoxin, and zearalenone (ZON).[13] Animal feed containing grain contaminated with DON and ZON can have adverse health effects in swine, cattle, poultry and horses, including feed refusal, weight loss and abortion.[14].

Currently, management of WBC includes the use of transgenic (i.e. *Bt*) corn hybrids, or the use of monitoring, scouting and action thresholds to apply insecticides by ground or aerial sprays when necessary.[4] Although there are few fungicides available for use in managing ear rot fungi, with limited efficacy data, fungicides have become common inputs in corn production to manage foliar diseases. Among the most commonly applied fungicides on corn are the quinone-outside inhibiting (QoI) fungicides [Fungicide Resistance Action Committee (FRAC) Group 11], commonly referred to as strobilurins. While fungicides can be applied to reduce or prevent spore germination and mycelial growth of some fungi, the efficacy of fungicides to reduce severity of *F. graminearum* infection and the resulting mycotoxin production has not been consistent, and applications of QoI fungicides to wheat at anthesis have been reported to result in increased DON production in grain.[15] Therefore, the efficacy of QoI fungicides on *F. graminearum* infection and mycotoxin development is an area that requires further study.

The husk of the corn ear forms a barrier around kernels, protecting developing ears from damage. When the husk is compromised by insect feeding, the risk of fungal infection increases; this may result in decreased yields in infested fields.[16] Several published field studies have shown that insect damage to corn ears and stalks is correlated with an increase in disease presence,[17–19] although in some cases no relationship has been documented.[20,21] Caterpillars that feed on corn plants, such as the European corn borer (*Ostrinia nubilalis* Hübner) and corn earworm (*Helicoverpa zea* Boddie), facilitate infection by damaging host plants and acting as vectors of fungal pathogens, increasing the incidence of ear and stalk rots.[17,22–24] Parsons and Munkvold[17] determined that Fusarium ear rot occurrence, caused by *F. verticillioides*, correlates directly with higher populations of ear-infesting insects, such as thrips (*Frankliniella* spp.) and corn earworm.

Because of these previously demonstrated relationships between insect feeding and disease development and their occupying a common niche in the corn ear, we hypothesized that feeding damage from WBC larvae may be of concern beyond losses in yield caused by consumption of ears. The primary objective of this study was to determine whether WBC larval feeding damage exacerbated ear rot development in field corn and resulted in increased mycotoxin levels. The effect of a QoI fungicide application on Gibberella ear rot severity and DON production, both with and without WBC infestation, was also evaluated.

2 EXPERIMENTAL METHODS

2.1 Field trials

Research trials were conducted at the Throckmorton Purdue Agricultural Center (TPAC) near Lafayette, Indiana, during 2011 and 2012. The commercial hybrid 32 T82 (Pioneer Hi-Bred International, Johnston, IA), treated with Cruiser Extreme 250, was planted with Force 3G® (tefluthrin; Syngenta AG, Basel, Switzerland) for rootworm protection at a rate of 113.4 g per 304.8 row meters. Seeds were planted using a four-row planter (John Deere MaxEmerge 7000, drawn planter) with row spacing at 0.76 m with standard spacing between plants (approximately 16.5 cm). Planting speed was 4.8 km h^{-1}, and a dry starter fertilizer was planted in a 12-12-12 (N-P-K) ratio at 51.3 kg ha^{-1}.

Each year the experiment was arranged in a randomized complete block design with four replicates (i.e. plots) per treatment. Plots measured 29 m × 3 m, with 1.5 m alleys in-between plots. Four border rows were planted between this study and adjacent fields. Planting took place at a typical planting time for the region, on 12 May and on 4 May in 2011 and 2012 respectively. Eight treatments were implemented in 2012 in a 2 × 2 × 2 full factorial design, as follows: (1) non-treated control (not infested with WBC, not inoculated with *F. graminearum* and not sprayed with fungicide); (2) WBC infested; (3) *F. graminearum* inoculated; (4) application of the QoI fungicide pyraclostrobin (Headline®; BASF Corporation, Research Triangle Park, NC);[25] (5) WBC × *F. graminearum*; (6) WBC × pyraclostrobin; (7) *F. graminearum* × pyraclostrobin; (8) WBC × *F. graminearum* × pyraclostrobin. Six treatments were implemented in 2011 as follows: (1) non-treated control; (2) WBC infested; (3) *F. graminearum* inoculated; (4) pyraclostrobin alone; (5) WBC × *F. graminearum*; (6) *F. graminearum* × pyraclostobin.

2.1.1 *F. graminearum* inoculation

F. graminearum inoculum was prepared using a mung bean liquid medium method. To prepare the medium, 40 g of organic mung beans was added to 1 L of distilled water.[26] The mixture was allowed to steep for 15 min at 97 °C, after which the beans were removed from the liquid by straining the mixture through two layers of cheesecloth. The strained liquid was autoclaved and inoculated with one of five separate *F. graminearum* (anamorph of *G. zeae*) isolates collected in Indiana. The suspensions were kept on a rotating shaker (110 rev min^{-1}) for 10 days under a 12 h light, 12 h dark cycle at 25 °C. After 10 days the conidial suspensions in each of the five flasks were quantified using a hemocytometer, and the concentrations were adjusted to 5×10^4. The adjusted suspensions were then mixed together equally for use in field experiments.

Prepared inoculum was injected into each ear in the middle two rows of each research plot receiving an *F. graminearum* inoculum treatment at growth stage R1 (early silking), as this approach has been shown to result in consistent disease severity.[27] Inoculum was delivered directly into the silk channel of the ear at a volume of 5 mL and a concentration of 5×10^4 conidia mL^{-1}, via a 60 mL syringe with an 18 gauge 3.8 cm blunt end needle (BD, Franklin Lakes, NJ) in 2011, or a modified 2 L Outdoor Products hydration backpack (Outdoor Products, Los Angeles, CA) attached via vinyl tubing to a 5 mL Allflex 5 EM-B ultra-automatic precision syringe (Allflex USA, Inc., Dallas/Fort Worth, TX) tipped with a blunt 18 gauge 3.8 cm needle in 2012. At each inoculation timing, the needle tip was inserted into the ear via the silk channel as far as possible before injecting the inoculum. Inoculation took place beginning at approximately 7:00 a.m. on 19 July 2011 and 13 July 2012.

2.1.2 *Fungicide application*

One day prior to *F. graminearum* inoculations, the pyraclostrobin fungicide Headline was applied over the canopy of plots at a rate of 0.437 L ha^{-1} using a CO_2 pressure driven backpack sprayer with a 1.52 m wide handheld boom fitted with four TJ-VS 8001 nozzles spaced 50.8 cm apart, which delivered 140.3 L ha^{-1} at 2.81 kg cm^{-2}.

2.1.3 Western bean cutworm infestation

Artificial infestation of plots with WBC larvae was done by collecting egg masses from commercial cornfields in Jasper, Newton and LaPorte counties in Indiana. Eggs were reared in incubators programmed to reflect natural settings (variation in temperatures between hours of day and night). The programmed settings were 22.5–23.0 °C for daylight hours and 15 °C during nighttime hours with 80% relative humidity. Larvae were fed using fresh corn silks, tassels and kernels with moistened cotton swabs every 2 days while silks and tassels were still available from nearby fields. When larvae reached the second and third instar, two larvae were transferred into 21 mL plastic portion cups (Gordon Food Services, Grand Rapids, MI) with lids that had 2 mm holes in them for ventilation. After 7–10 days, larvae were individually transferred to 227 mL plastic cups (Dixie Dessert Dishes; Dixie Consumer Products LLC, Atlanta, GA) where they were maintained for approximately 14 days. During this time, larvae were fed with chunks of corn ears obtained from the experimental plot at TPAC, with the cob leaves still intact. These ears were not treated with any foliar pesticides during the growing season and were a non-*Bt* commercial hybrid. Cups were washed with a mixture of water and Alconox Powdered Precision Cleaner (Alconox, Inc., White Plains, NY) and rinsed 3 times every fourth day to remove frass and mold. Plants within the center two rows of treatments were infested with WBC larvae at corn growth stage R2 (blister stage) with instars ranging from third to fifth. Ears were flagged approximately 6 m from the beginning of the plot and infested by parting selected silks and depositing two larvae within the silks, close to the developing ear. Every fourth plant within the row was infested with a total of 20 infested plants per WBC-treated plot (ten per row) on 28 July in 2011. Owing to a scarcity of egg masses for infestation during 2011, the WBC × pyraclostrobin and WBC × *F. graminearum* × pyraclostrobin treatments were not infested. On account of poor egg hatch in 2012, the protocol was altered slightly, and plants were infested multiple times as follows: plants in replicate 1 were infested on 27 June (three egg masses per treatment) and again on 2 July 2012 (three egg masses per treatment). Replicates 2 to 4 were infested with six egg masses per treatment on 9 July 2012. Egg masses were attached, along with an approximately 1 cm area of the surrounding corn leaf, to the newest leaf adjacent to the whorl using a single staple; this is the area preferred by ovipositing female moths.

2.2 Data collection

Ears were collected at two separate times during the growing season in 2011 and on 12 October 2012. The first ear collection occurred on 25 August 2011 to evaluate western bean cutworm larval feeding damage, and the second collection occurred on 17 October 2011 to assess symptoms of Gibberella ear rot. Ears were collected from treatments 1 (control), 2 (WBC) and 5 (WBC × *F. graminearum*) for western bean cutworm larval feeding damage measurements. In treatment 1 (control), 37 ears were collected. Walking approximately 3 m in-between rows 2 and 3, every fourth ear from row 2 was collected until 18 ears were collected in total. Ear collection from row 3 was then started, harvesting every fourth ear until 19 ears were collected, resulting in a total of 37 ears. Ears were placed in paper bags marked with the rep and the treatment. In treatment 2 (WBC only), 74 ears were numbered and collected. Ears infested with western bean cutworm larvae in rows 2 and 3 were harvested and bagged (numbers 1–20) with a label containing the rep, treatment and the word infested or 'I'. After the infested ears were collected, ears that were not manually infested with larvae were collected from the same plots, marked (21–74), bagged and labeled with the rep, treatment and the word uninfested/non-infested or 'U'. For assessment of Gibberella ear rot severity, all ears between the first and last infested ear in the two center rows of each plot were collected on the dates listed above. Husks were removed from ears, and ears were stored in labeled paper or mesh bags at a temperature of 5.5 °C until ratings could be completed.

Quantitative ratings of ear damage were completed using transparency film with a grid composed of 1 cm × 1 cm squares (Avery Office Products, Brea, CA). The film was wrapped around each ear, and total width (around the base of the ear) and length of the ear were recorded. The total area damaged by larval feeding and the area colonized by *F. graminearum* (mycelial growth) were also calculated to assess insect damage and disease severity. From these values, percentage ear rot severity and insect damage were calculated as a proportion of total ear area. In 2011, disease severity assessments were based on visual identification of causal organisms based on plant symptoms and fungal signs presented. In 2012, environmental factors confounded disease severity ratings. Drought conditions resulted in multiple fungal pathogens present on sampled ears, including *Aspergillus flavus* and *Penicillium* spp. Therefore, a general ear rot rating was used that combined the total ear rot damage caused by all pathogens present.

2.2.1 Deoxynivalenol quantification

In both years of the experiment, individual ears were shelled and grain from treatments was ground in a Romer Series II Mill (Romer Labs, Inc., Union, MO), and grain was combined into one bulk sample per plot. The sheller and mill were thoroughly cleaned between shelling and grinding of individual plots to prevent contamination of samples. Grain from each plot was mixed thoroughly after the grinding process, and 454 g was removed for DON testing. The Envirologix QuickTox Kit for QuickScan DON in corn and wheat (cat. no. AQ 204 BG; Envirologix, Portland, Maine) was used to assess DON concentrations within individual plots (range of detection 200–10000 ppb). A quantity of 20 g of the 454 g grain sample from each plot was mixed with 100 mL of tap water (5 mL for every gram of sample) in a 150 mL disposable plastic cup, and shaken vigorously for 30 s.

The grain sample was then allowed to settle into layers. During the settling period, a reaction vial was prepared for sample analysis by pipetting 800 μL of buffer (provided) into the reaction vial. The pipette tip was discarded and, with a new pipette tip, 200 μL of the grain sample was pipetted into the vial. The mixture was stirred with the pipette tip, and 200 μL was removed and added to a new vial. A QuickTox strip was then placed in the vial containing the 200 μL mix of buffer and extract and allowed to stand for 10 min. After the reaction time had passed, the QuickTox strip was scanned using an Envirologix Quickscan Strip Reader (Model #ACC131) to measure DON levels within the grain sample. The QuickTox strips had a lower detection limit of 0.2 ppm and an upper detection limit of 10 ppm. If DON levels in the samples exceeded 10 ppm, additional dilutions were performed until an endpoint value was reached.

2.2.2 Data analysis

Ear rot and mycotoxin data were analyzed using Statistica 12 (Statsoft, Inc., Tulsa, OK) for each year of the study. Quantitative data

(including DON concentration data) were analyzed using analysis of variance, with treatment as the independent variable categorized by the presence/absence of each factor, and disease severity as the dependent variable. While a full factorial design was implemented in 2012, this was not possible in 2011 owing to scarcity of WBC egg masses. Non-transformed data were analyzed in both years because a square root transformation did not improve homogeneity of variance.

3 RESULTS

3.1 The 2011 field experiment

In 2011, we found no significant interactions among treatments with regard to disease severity. Rather, the presence of *F. graminearum* was the only significant factor influencing disease severity ($F_{1,18} = 24.56$, $P < 0.001$). Corn ears that were inoculated with *F. graminearum* exhibited much higher incidence of disease than corn ears that were not inoculated (Fig. 1). Analysis of WBC feeding damage on ears from 2011 revealed that damage was significantly higher in treatments infested with WBC larvae ($F_{1,18} = 35.77$, $P < 0.001$) (Fig. 1).

All grain samples tested for total DON in 2011 indicated that detectable DON was below the lower limit of detection of 0.25 ppm for the mycotoxin analysis kit (data not shown).

3.2 The 2012 field experiment

There was a significant three-way interaction between WBC, *F. graminearum* inoculation and fungicide application on disease severity in 2012 ($F_{1,16} = 19.23$, $P < 0.001$). In the absence of fungicide treatment, corn ears exhibited greater disease severity when they were artificially inoculated with *F. graminearum* and exposed to WBC (Fig. 2). Conversely, when fungicide applications were made, corn ears exhibited greater disease severity when they were artificially inoculated with *F. graminearum* and WBC was absent (Fig. 2).

In 2012 there was also a significant three-way interaction between WBC, *F. graminearum* inoculation and fungicide treatment on corn ear damage ($F_{1,16} = 5.74$, $P = 0.03$). When no fungicide application occurred, damage to corn ears was lowest in treatments inoculated with *F. graminearum* but not exposed to WBC. While damage was greater in the remaining treatments, there were no significant differences in damage levels with regard to *F. graminearum* inoculation or WBC (Fig. 3). However, when fungicide was applied, less ear damage due to WBC was observed in treatments inoculated with *F. graminearum*, such that damage was lower when both WBC and *F. graminearum* were present than when WBC was present without *F. graminearum* inoculation (Fig. 3).

DON levels were significantly different among treatments for analyzed grain samples in 2012 ($F_{\text{treatment (7,48)}} = 88.20$, $P < 0.001$; $F_{\text{rep (2,48)}} = 86.86$, $P < 0.001$). Treatments that had similar DON levels were: WBC alone and pyraclostrobin alone; *F. graminearum* × pyraclostrobin and the non-treated control; control and WBC × pyraclostrobin; WBC × *F. graminearum* × pyraclostrobin, *F. graminearum* and WBC × *F. graminearum* (Fig. 4).

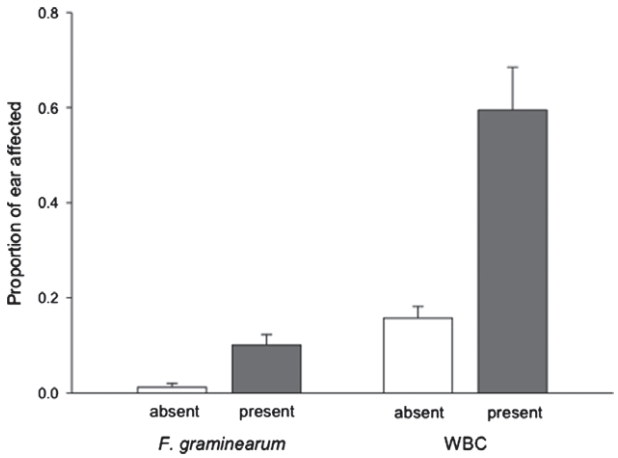

Figure 1. Effect of *F. graminearum* inoculation and WBC infestation upon disease severity and damage, respectively, in 2011. *F. graminearum* inoculation showed a main overall effect of treatment ($F_{5,18} = 8.16$, $P = 0.0004$), with the presence of *F. graminearum* as the only significant factor ($F_{1,18} = 24.56$, $P = 0.0001$). WBC infestation showed a main overall effect of treatment ($F_{5,18} = 14.28$, $P < 0.0001$), with the presence of WBC as the only significant factor ($F_{1,18} = 35.77$, $P < 0.0001$).

4 DISCUSSION AND CONCLUSIONS

At the outset of this study, it was hypothesized that the presence of WBC larvae and feeding damage on ears would encourage *F. graminearum* colonization of ears, thus increasing ear rot severity. Indeed, treatments of WBC larvae and *F. graminearum* consistently resulted in higher disease severity than all other treatments in the 2011 field experiments. In 2012, the combination treatment resulted in higher disease levels than WBC larvae alone. Previous work exploring relationships between corn pests and fungal pathogens have found similar trends. One study found that ears lacking damage of corn earworm larvae had a 7% rate of *Fusarium* infection compared with 26.5% in ears with larval damage.[28] Non-transgenic corn hybrids have been documented with higher levels of the mycotoxin deoxynivalenol (DON) than transgenic corn in cases where lepidopteran pests were present, and this is attributed to the protection provided by the insect resistance traits.[29–31] These studies, and the results we present here, support the notion that effective management of ear rot pathogens should start with management of ear-feeding pest insects. In current production systems, this is often accomplished through the use of *Bt* hybrids that express one or more proteins targeting ear- and stalk-feeding pests;[31] these hybrids have been readily adopted and now comprise over 85% of all US corn planted.[32] The work we describe here underscores the importance of resistance management in preserving the durability and effectiveness of these tools for management of WBC and other ear-feeding pests.

Interestingly, there were no differences in disease severity among treatments that included an application of the QoI fungicide pyraclostrobin, suggesting that an application of pyraclostrobin fungicide had no impact on suppressing the development of ear rot disease. The lack of fungicide efficacy observed against Gibberella ear rot is not altogether surprising, as this class of fungicides has limited translocation/mobility within the plant and may not be able directly to penetrate the husk tissue where fungal growth occurs. A similar lack of consistent fungicide efficacy for ear rots has been observed in other pathosystems, including Diplodia ear rot caused by *Stenocarpella maydis*.[33] A fungicide treatment was included in our experiments because QoI fungicides are prevalent in current production systems as foliar fungicides, and it was important to test its ability to increase DON when applied during reproductive stages of corn, as is observed in wheat. The increase in DON that occurs as a result of a QoI fungicide application in wheat has been attributed to

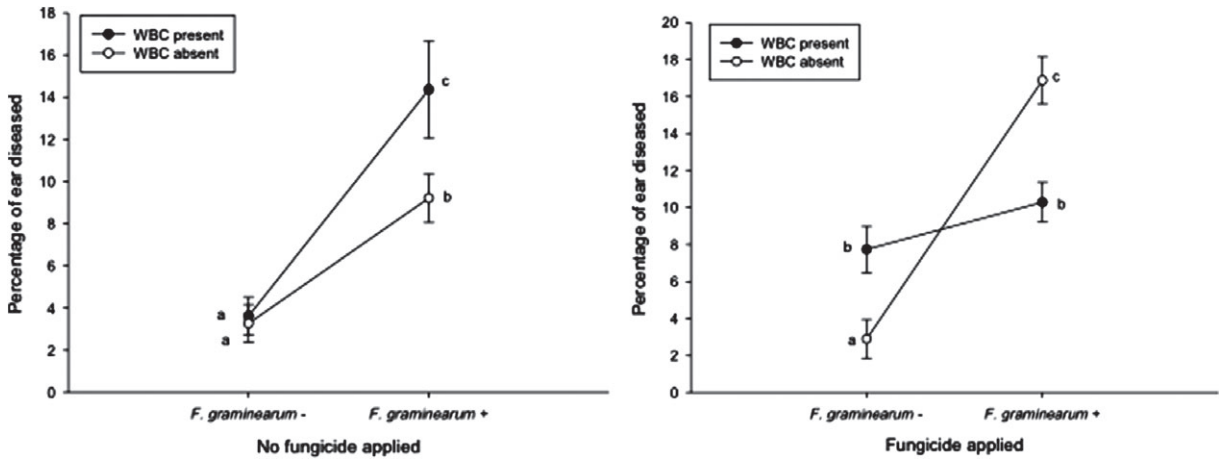

Figure 2. Effect of interaction between factors upon disease severity in 2012, showing that there was a significant three-way interaction between WBC, *F. graminearum* and fungicide on disease ($F_{1,16} = 19.23$, $P = 0.0005$). When fungicide was absent, disease severity was enhanced by WBC in the presence of *F. graminearum*. However, when fungicide was applied, disease severity was greater in treatments that were inoculated with *F. graminearum* but lacked WBC.

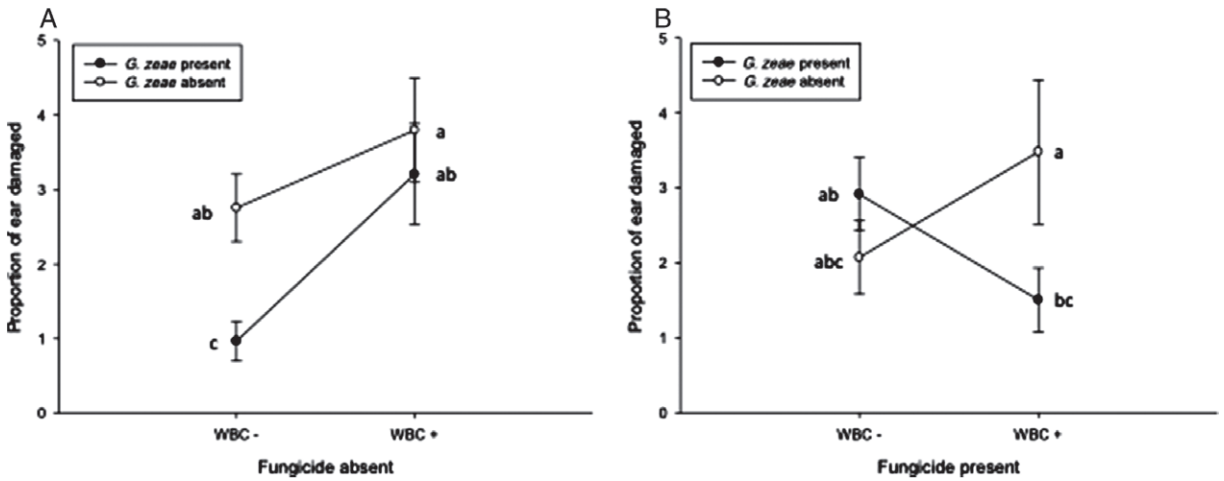

Figure 3. Effect of interaction between factors upon ear damage in 2012, showing that there was a significant three-way interaction between WBC, *F. graminearum* and fungicide on damage ($F_{1,16} = 5.74$, P = 0.02). When fungicide was absent, corn ear damage was greatest in treatments containing WBC with no *F. graminearum*. However, when fungicide was applied, the presence of *F. graminearum* reduced damage by WBC.

elimination of non-pathogenic microorganisms that may allow *F. graminearum* to colonize tissue freely,[34,35] or to the delayed senescence that occurs with applications of QoI fungicides, which gives the fungus a longer timeframe for colonization and mycotoxin production.[36,37] However, in our study a QoI fungicide application did not increase DON in grain in 2011 or 2012.

In 2012, the experimental location experienced severe drought conditions[38] that impacted results and were unfavorable for Gibberella ear rot development.[39] In 2012, WBC × *F. graminearum* and *F. graminearum* alone treatments exhibited significantly less ear rot than the *F. graminearum* × pyraclostrobin treatment. In previous years of the experiment, treatments infested with WBC had the highest levels of disease, and therefore it was somewhat unexpected that the treatment with the highest ear rot rating in 2012 was not infested with WBC larvae. However, this is likely due to the observation that in 2012 every plot had some degree of caterpillar feeding damage [corn earworm, *Helicoverpa zea* (Boddie)], whereas only infested treatments in 2011 exhibited significant feeding damage. Corn earworm infestations in field corn are uncommon and sporadic in our study area, and no other caterpillar feeding was noted in 2011. Analysis of feeding damage revealed that feeding damage in the 2012 *F. graminearum* × pyraclostrobin treatment was visually similar to damage observed in WBC treatments in prior years (i.e. feeding on ear tips and surface scraping of kernels). These results may indicate that *F. graminearum* and other fungi colonize ears more readily when any lepidopteran pest feeding damage occurs, as opposed to a specific relationship between WBC and the pathogen.

A second counterintuitive result in 2012 was the observation that the non-inoculated WBC × pyraclostrobin treatment had similar levels of ear rot to the inoculated *F. graminearum* alone treatments. It was expected that treatments that were not inoculated with *F. graminearum* would exhibit lower disease severity, based on results obtained in 2011. However, the drought conditions greatly favored growth and infection of several fungal species, including *A. flavus*, *F. verticillioides* and *Penicillium* sp., which were visually observed on sampled ears in the plots.[40–42] The implications of this finding are that *F. graminearum* was not the only pathogen present in fields, and that WBC encourages colonization of damaged ears by many pathogens, not just *F. graminearum*.

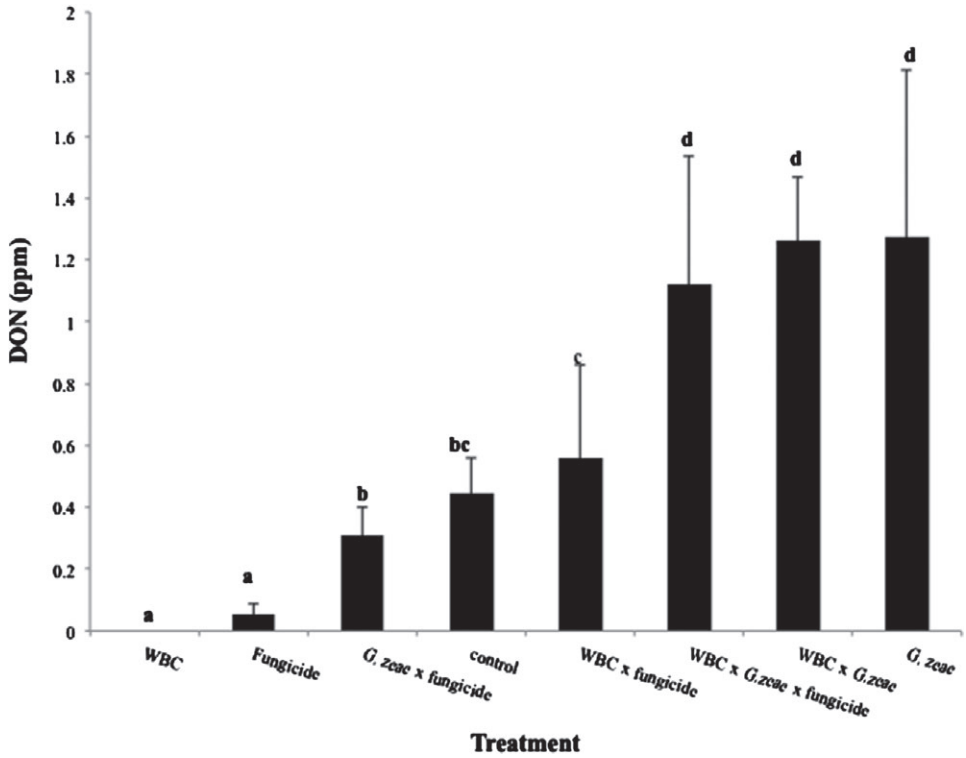

Figure 4. Deoxynivalenol (DON) concentrations (ppm) from treatments in the field for the 2012 field season. Treatments consisted of a control, western bean cutworm (WBC), *Gibberella zeae*, fungicide [Headline® (pyraclostrobin)], WBC × *G. zeae*, WBC × fungicide, *G. zeae* × fungicide and WBC × *G. zeae x* fungicide. Means and standard errors are shown. Bars with different letters assigned were found to be significantly different ($F_{7,48} = 88.20$, $P < 0.0001$, $n = 72$).

Mycotoxin analyses from 2011 and 2012 demonstrated that DON concentrations in grain varied by year. DON levels were higher from grain sampled in 2012 than in 2011, in spite of the lower visual levels of *F. graminearum* on ears in 2012. These results are not unexpected, as mycotoxin levels vary greatly in grain. Whitlow *et al.*[43] and Coulombe[44] have observed that the prevalence and concentrations of mycotoxins are different from year to year, depending on environmental factors. Mycotoxin levels and visual ear rot severity are not always correlated, and DON is difficult to correlate with disease levels in similar pathosystems, such as wheat.[44,45] Producers and pest managers should be aware of the environmental conditions within fields in order to assess and prioritize risks to grain quality. The presence of mycotoxins in corn grain can decrease the quality, marketability and storability of contaminated grain, which results in lost income to producers, and the contaminated grain is potentially hazardous to humans and livestock. Common toxicity symptoms in livestock include feed refusal, vomiting and hormonal and reproductive issues.

During the course of this study, corn supply and demand increased steadily.[2] As a result of this trend, grower acceptance of risk has declined, and the dual application of insecticides and fungicides has become an increasingly common practice in both corn and soybean in the Midwest.[46,47,48] This offers the benefits of applying multiple prophylactic pesticides simultaneously, reducing application costs. However, it is important to time insecticide applications properly, and it is advisable to apply chemical pesticides to corn plants before larvae migrate to ears, as efficacy of these contact insecticides will decline dramatically once larvae have entered developing ears.[4] Although fungicides are commonly applied to corn along with insecticide applications, the results from each year of our study indicate that one of the most commonly used fungicides in corn production, pyraclostrobin, does not suppress Gibberella ear rot severity. Additional research is needed to assess whether insecticide applications that reduce WBC or other ear-feeding pest severity can indirectly reduce Gibberella ear rot severity; the results from the present study suggest that this is a recommendation worth exploring further for WBC and other serious ear-feeding caterpillar pests of corn.

Our results parallel those of others examining WBC and its association with ear rots. In one study,[49] Bowers *et al.* found similar trends, whereby greater kernel injury attributed to WBC (and other ear-feeding Lepidoptera) led to increased levels of Fusarium ear rot and subsequent higher levels of fumonisins in grain. Importantly, both of these studies were focused chiefly upon quantifying *Bt* hybrid treatment differences with respect to the presence of *Cry* toxins targeting lepidopteran pests, and in both cases it was demonstrated that targeting larvae with these hybrids effectively reduced the incidence of the disease and mycotoxins. Gibberella ear rot was the focus of these studies, but it is worth noting that WBC infestations may increase the severity of other ear rot diseases, as several fungal pathogens enter corn ears through wounds, as well as silks. Like many pests of annual crops, WBC pressure levels can be reduced by an IPM program that includes crop rotation whenever possible. In areas where WBC is a frequent pest, use of transgenic corn hybrids (i.e. *Bt* corn) that protect against this and other pests clearly provides an effective first line of defense. However, in many of the States where WBC is a relatively new pest (including Indiana), infestations are confined to relatively small geographic areas and have thus far been sporadic.[4] Furthermore, resistance to these in-plant toxins may eventually

force producers in some locations to seek other control options. In these areas, appropriately timed insecticide applications, based on monitoring and scouting programs, can effectively manage WBC infestations and reduce the risk of Gibberella ear rot and associated mycotoxin development in corn.

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