TRANSGENIC PLANTS AND INSECTS

A Comparison of Bt Transgene, Hybrid Background, Water Stress, and Insect Stress Effects on Corn Leaf and Ear Injury and Subsequent Yield

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ABSTRACT Experimentally manipulated water and insect stresses were applied to field-grown corn with different *Bacillus thuringiensis* (Bt) transgenes and no Bt transgenes, and different nontransgenic hybrid backgrounds (2011 and 2012, Corpus Christi, TX). Differences in leaf injury, ear injury, and yield were detected among experimental factors and their interactions. Under high and low water stress, injury from noctuid larvae (Lepidoptera: Noctuidae) on leaves during vegetative growth (primarily from fall armyworm, *Spodoptera frugiperda* J.E. Smith) and on developing ears (primarily from corn earworm, *Helicoverpa zea* [Boddie]) was lowest on more recent releases of Bt hybrids (newer Bt hybrids) expressing Cry1A.105+Cry2Ab2 and Cry 3Bb1, compared with earlier Bt hybrids (older Bt hybrids) expressing Cry1Ab and Cry3Bb1 and non-Bt hybrids. High water stress led to increased leaf injury under substantial fall armyworm feeding pressure in 2011 (as high as 8.7 on a 1–9 scale of increasing injury). In contrast, ear injury by corn earworm (as high as 20 cm^2 of surface area of injury) was greater in low water stress conditions. Six hybrid backgrounds did not influence leaf injury, while ear injury differences across hybrid backgrounds were detected for non-Bt and older Bt hybrid versions. While newer Bt hybrids expressing Cry1A.105+Cry2Ab2 and Cry 3Bb1 had consistent low leaf injury and high yield and low but less consistent ear injury across six hybrid backgrounds, water stress was a key factor that influenced yield. Bt transgenes played a more variable and lesser role when interacting with water stress to affect yield. These results exemplify the interplay of water and insect stress with plant injury and yield, their interactions with Bt transgenes, and the importance of these interactions in considering strategies for Bt transgene use where water stress is common.

KEY WORDS fall armyworm, corn earworm, *Spodoptera frugiperda*, *Helicoverpa zea*, Bt corn

Fall armyworm, *Spodoptera frugiperda* J.E. Smith, and corn earworm, *Helicoverpa zea* (Boddie; Lepidoptera: Noctuidae), are important leaf- and ear-feeding pests of corn, *Zea mays* (L.). Yield decline may result from larval feeding on leaves during vegetative growth and kernels of developing ears (Curly 2000). In the southern United States, fall armyworm and corn earworm infest field corn from whorl stage through ear development (Curly 2000, Buntin 2008). In addition, water and heat stress may reduce corn yield directly and indirectly through their effects on insect pests (Haile 2000). As one example, the southern United States has been recently experiencing frequent episodes of low rainfall and high temperatures, which adversely affected corn production (Baumhardt and Salinas-Garcia 2006). Crop plants under water stress have been observed to be more prone to insect injury and adversely affect yield (Haile 2000), and the form of the water stress and mode of insect feeding may affect insect performance on water-stressed plants (Huberty and Denno 2004).

Transgenic field corn expressing insecticidal proteins derived from *Bacillus thuringiensis* (Bt) Berliner has been used to control these and other insect pests. Hybrids with Bt transgenes recently constituted ≈76% of corn planted within the United States (USDA ERS 2014), Europe, South America, and elsewhere (James 2012). Over several decades of development, Bt transgenes incorporated into corn have progressed from hybrids with a single Bt transgene to the more recent releases of Bt hybrids with two or more Bt transgenes. Corn hybrids with earlier releases of Bt transgenes included those with the transgene expressing Cry1Ab protein in event Bt11 (Syngenta Seeds, Raleigh, NC) and event MON810 (Monsanto Co., St. Louis, MO). Bt hybrids with Cry1Ab had high efficacy against stalk-boring lepidopteran corn pests (e.g., European corn borer, *Ostrinia nubilalis* (Hubner); Hutchison et al. 2010). They also reduced to varying degrees, leaf and ear injury caused by fall armyworm and corn earworm (i.e., 50–90% leaf and ear injury reduction compared with non-Bt counterparts), but the reduced injury corresponded to variable yield benefits (Horner et al. 2003, Buntin et al. 2004, Chilcutt et al. 2006, Buntin

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2008). Corn hybrids with more recent releases of Bt transgenes (e.g., the transgene expressing Cry1A.105 + Cry2Ab2 proteins in event MON89034; Monsanto Co., St. Louis, MO) had increased effectiveness against these species (Siebert et al. 2012).

Further adoption of Bt corn in the southern United States may be warranted now that control of fall armyworm and corn earworm has improved with the newer Bt transgenes. However, effects on leaf and ear injury and subsequent yield may be complex where combinations of abiotic (e.g., episodes of droughty conditions) and biotic (e.g., infestations of leaf- and ear-feeding insects) stresses occur in the southern United States and elsewhere (Haile 2000, Buntin 2008). The response of hybrids with different genetic background including Bt transgenes to such abiotic stress may warrant consideration in adoption of Bt corn hybrids to control leaf- and ear-feeding insects. Experimentally manipulated water and insect stresses were applied to field-grown corn with different hybrid backgrounds and Bt transgene packages (including non-Bt versions) to better characterize and compare these effects on leaf and ear injury and subsequent yield.

Methods and Materials

Experimental Treatments and Design. A field experiment varied water and insect stresses across 18 corn hybrids, consisting of six hybrid groups each with a non-Bt hybrid and two Bt hybrids differing in Bt transgene packages. Insect and water stresses were manipulated by using foliar insecticides and drip irrigation on field corn planted at a site typically experiencing high insect and water stresses. The experiment was conducted in the southern edge of the coastal corn-growing region of south Texas, in Corpus Christi, TX, during two growing seasons, 2011 and 2012. The experimental setting was ≈2.4 ha in size, which was rotated yearly with cotton. The soil type was an Orelia sandy-clay loam. Fall armyworm regularly occurred and outbreaks were episodic, while corn earworm infestations regularly resulted in high infestations of ears. In contrast, very little feeding pressure from stalk borers and stink bugs was observed (M.J.B. and D.J.A., personal observation), and Mexican corn rootworm, *Diabrotica virgifera zeae* Krysan & Smith (Coleoptera: Chrysomelidae), pressure was minimized using the standard practices of planting insecticide-treated seed and crop rotation (Porter et al. 2005). These conditions allowed experimental focus on plant injury caused by fall armyworm and corn earworm. The site also experienced multiyear high temperatures and low rainfall from 2011 to 2013, classified as severe to exceptional drought (National Weather Service 2012). To maximize natural fall armyworm and corn earworm incidence, corn was planted late: 18 April in 2011 and 16 April in 2012. Plant stand was 44,500–49,500 plants per ha. No foliar insecticides and irrigation were used other than as prescribed in the insect and water stress protocols. Roundup herbicide (Monsanto Co., Saint Louis, MO) was used for weed control on the hybrids in the experiment, which were all glyphosate-tolerant. No foliar fungicides were applied.

The treatment combinations of water stress (two levels), insect stress (two levels), hybrid group (six levels), and Bt transgene status (three levels) were set out in a split-split-split plot design using two-row plots (7.9 m row length, 0.97 m row width). The 72 treatment combinations were replicated six (2011) and five (2012) times. The water stress factor was randomized in the main plots (two levels: relatively low water stress and high water stress achieved with irrigation supplementing very low rainfall). The nonwater stress plots received rainfall and irrigation to achieve nonlimiting water conditions targeting ≈90% crop evapotranspiration replacement. Irrigation in water stress plots also was needed to achieve moderately severe limiting water conditions targeting ≈50% crop evapotranspiration replacement. Soil moisture probes (Watermark probes, Irrometer, Riverside, CA) were placed in the two water stress conditions in each main plot, set at 0.3 and 0.6 m depths (Alam and Rogers 1997) midway between irrigation drip emitters. From planting to 2 weeks after first silk, irrigation was initiated when soil moisture tension approached 40 centibars on average from the 0.3-m-deep probes (Alam and Rogers 1997) and when weather forecasts indicated no imminent rainfall. Using drip irrigation combined with the sandy-clay soil type minimized soil moisture encroachment across adjacent dryland plots.

The insect stress factor (two levels: low insect stress achieved with foliar insecticide application, and high insect stress with no insecticide use) was the first split in the experimental design. A high-boy sprayer with an extension boom was used to spray zeta-cypermethrin (Mustang Max, FMC Co., Philadelphia, PA) and rynaxpyr (Coragen, DuPont Co., Wilmington, DE; tank mix) in 2011 and rynaxpyr in 2012 in the low insect stress plots. One whorl-stage spray and twice-weekly sprays during silking were applied at labeled rates in 280 liters of water per ha using flat fan nozzles (Colorjet RF3, Delavan AgSpray Products, Mendota Heights, MN) to maximize coverage. Sub-blocks of the insect stress factor were bordered by one row of a non-Bt hybrid, and sub-block pairs were separated by six nonplanted rows. This design allowed plot access for the sprayer, reduced spray drift, and further separated low and high water stress plots.

The hybrid group factor (six hybrid groups) was the next split in the experimental design. The six hybrid groups differed in nontransgenic breeding background and represented a range of hybrids from five commercial seed companies using several Bt transgene events (Monsanto Co., St. Louis, MO), all with a transgene expressing glyphosate resistance and with corn hybrid maturity ratings of 110–114 d. The final split in the design was Bt transgene factor represented by three near-isogenic hybrids of each hybrid group (three levels: two hybrids with different Bt transgene packages and a non-Bt hybrid version in each hybrid group). The specific Bt treatments represented in the hybrids were 1) no Bt transgenes (non-Bt hybrid), 2) an earlier release of two Bt transgenes expressing

Cry1Ab protein in event MON810 and Cry3Bb1 protein in event MON88017 (older Bt hybrid), and 3) a more recent release of the combination of transgenes expressing Cry1A.105 + Cry2Ab2 proteins in event MON89034 and Cry3Bb1 protein in event MON88017 (newer Bt hybrid). The varied genetic background of the six hybrid groups allowed interpretation of the results more broadly across an array of hybrid groups containing these Bt transgenes, instead of limiting interpretation to specific hybrids. All factor levels were randomized within their respective split plots of the experimental design. A fill range of corn was planted in the front and back edge of the plots as buffer from road effects.

Plant Injury Measurements. One row of each two-row plot was reserved for in-season plant injury evaluations, and the other row was reserved for harvest. Foliage was inspected for larval activity beginning at whorl stage. When fifth instars and leaf injury were prevalent, leaves of 20 plants from the in-season evaluation rows were rated for leaf injury using a 1–9 scale, ranging from 1 (=no visible injury or small pin holes visible on a few leaves) to 9 (=most of leaves with long lesions; Guthrie et al. 1960). Once fifth instars represented >90% of the larvae found during weekly inspection of ears from non-Bt corn border rows, the dominant ears of 20 plants from the in-season evaluation rows were hand-harvested and brought to the laboratory. Ear injury measurements taken were surface area of visible ear injury (tip + kernel injury) and length of visible larval penetration from entry point to the deepest point of feeding. In 2012, kernel injury was measured separately, which may be more directly related to yield (Curly 2000). For yield, all primary ears from the harvest rows were removed by hand after grain moisture reached 15% or lower, and the ears were stored in husk and dry storage to prevent postharvest kernel decline. Ears were threshed using an Almaco LPR thresher (Almaco, Nevada, IA), and grain yield was adjusted to 15% moisture content.

Experimental Analysis. An analysis of variance (ANOVA) conforming to the split-split-split plot design was used to assess effects focusing on interactions of water stress (two levels), insect stress (two levels), hybrid group (six levels), and Bt transgene (three levels) on leaf injury, ear injury, and yield. The leaf injury scale was transformed by the square root of the (count + 0.5) before analysis. The experimental design allowed strength in testing effects associated with the finer plot splits of Bt transgene and hybrid group and their interaction with water stress and insect stress (Neter et al. 1985). If interactions with Bt transgene were significant, contrast statements were used to compare responses for the non-Bt, older Bt, and newer Bt hybrids within each level of the interacting factor. Interactions with the hybrid group factor were used to evaluate response consistency across nontransgenic hybrid background. Further post-ANOVA mean comparisons among the six hybrid groups were not done, because pedigree information beyond incorporation of the transgenes was not available.

Regression relationships of yield (dependent variable) to the plant injury variables (independent variables) were further evaluated among experimental factors that were involved in significant interactions using indicator variables (also known as dummy variables; Neter et al. 1985, Chapter 10, Freund and Littell 2000). If the regression model was significant ($P < 0.05$) and informative ($R^2 > 0.20$), then indicator variables meeting model entry selection criteria ($P \leq 0.15$) were used to estimate separate regression lines. Yield response across the six hybrid groups tended to segregate in low and high yielding categories (see results); therefore, indicator variables were used to represent these two categories to avoid complexity in comparing many regressions lines (i.e., regressions of multiples of the six hybrid groups if interactions with the hybrid group factor were relevant; Neter et al. 1985).

Results

Fall armyworm and corn earworm were detected both years. Fall armyworm was the dominant leaf-feeder, representing ≈90% of larvae detected. Long feeding lesions occurred on about half the leaves on non-Bt hybrids (=7 leaf injury rating on average) in 2011, but leaf injury was limited, on average, to small shot-hole lesions on a few leaves in 2012 (=2 leaf injury rating on average). Corn earworm was the dominant ear-feeder, representing >95% of larvae detected. Ear injury was high on non-Bt hybrids in 2011 and modest in 2012 (as high as 9 and 6 cm^2 of surface area of ear injury on average, respectively). Average maximum temperatures of 35.3 and 35.1 °C from June through August were observed in 2011 and 2012, respectively. Rainfall from April through August was 5.84 cm in 2011 and 15.75 cm in 2012, compared with 45.7 cm in 2010 and a 35.5 cm average over 125 yr (Corpus Christi airport weather station 4 km from field site, National Weather Service 2012). Low water stress plots received 22.2 cm (2011) and 8.66 cm (2012) of irrigation water, and high water stress plots received 7.93 cm (2011) and 3.81 cm (2012) of irrigation water. In 2011, planting moisture was very poor, requiring irrigation in both water stress treatments to establish a good stand. However, subsoil moisture was excellent; therefore, the soil moisture probes worked well in timing water applications to achieve our desired water stress goals. Yield in the water stress plots ranged from ≈1,500 to 3,000 kg per ha in 2011, which was within the yield range for this dryland corn production region. In 2012, planting moisture was excellent, allowing good stand establishment. However, subsoil moisture was very poor due to the second consecutive year of drought, causing difficulties in obtaining good readings from the soil moisture probes and meeting the soil moisture targets. As a result, corn under low water stress produced yield about half the levels of those in 2011. Yields in high water stress plots were near zero; therefore, these data were not used in the analysis.

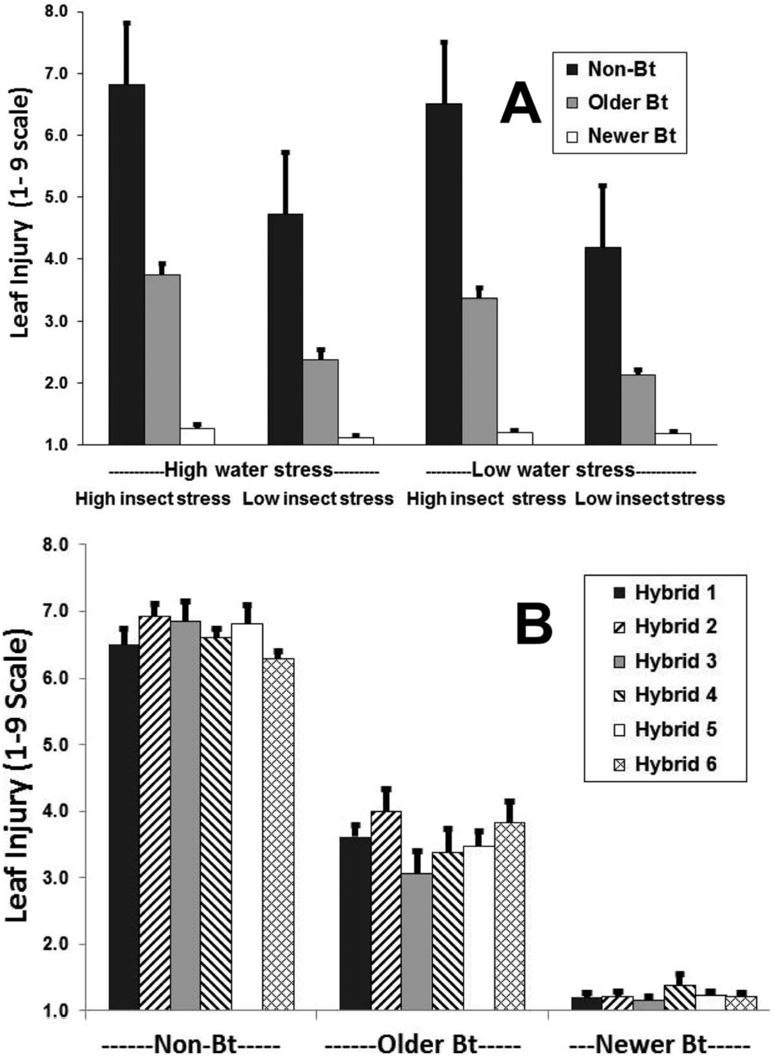

Fig. 1. Leaf injury (1–9 scale) means under high and low water stress and high and low insect stress for non-Bt hybrids, older Bt hybrids expressing Cry1Ab and Cry3Bb1, and newer Bt hybrids expressing Cry1A.105+Cry2Ab1 and Cry 3Bb2, averaged over six hybrid groups (A); and means of non-Bt, older Bt, and newer Bt versions of six hybrid groups, averaged over water stress and insect stress conditions (B). Lines are standard errors of the mean (SEMs), 2011, Corpus Christi, TX. Bt transgene interactions with insect stress and water stress (A) were detected ($P < 0.05$, see text for details).

Leaf Injury. In 2011, leaf injury differences and interactions were detected involving the experimental factors Bt transgene, insect stress, and water stress, but not hybrid group. The newer Bt hybrids had very low leaf injury under both high and low insect stress. In contrast, high insect stress intensified leaf injury in non-Bt and older Bt hybrids (Bt transgene by insect stress interaction: $F = 75.6$; df = 2, 240; $P < 0.0001$; linear contrast across non-Bt and Bt hybrids within the interaction: $F = 150$; df = 1, 240; $P < 0.0001$; Fig. 1A). To a lesser extent, high leaf injury occurred under high water stress for non-Bt and older Bt hybrids (Bt transgene by water stress interaction: $F = 3.10$; df = 2, 240; $P = 0.047$; linear contrast across non-Bt and Bt hybrids within the interaction: $F = 5.79$; df = 1, 240; $P = 0.017$; Fig. 1A). The effect of the Bt transgenes on leaf injury was consistent across hybrid groups (Bt transgene by hybrid group interaction not detected, $P = 0.13$; Fig. 1B), and no other two-way or higher-order interactions were detected ($P > 0.15$).

With less leaf injury occurring in 2012 (i.e., average injury was shot hole-type lesions on a few leaves), the only leaf injury differences detected were for the Bt transgene factor, and no interactions were detected ($P > 0.25$). Leaf injury on the non-Bt hybrids was greater than on either the older or newer Bt hybrids (quadratic contrast of the Bt transgene main effect: $F = 34.0$; df = 1, 190; $P < 0.0001$; Fig. 2, note change in scale from Fig. 1).

Ear Injury. In 2011, ear injury differences and interactions were detected involving all experimental factors measuring surface area of ear injury. Depth of

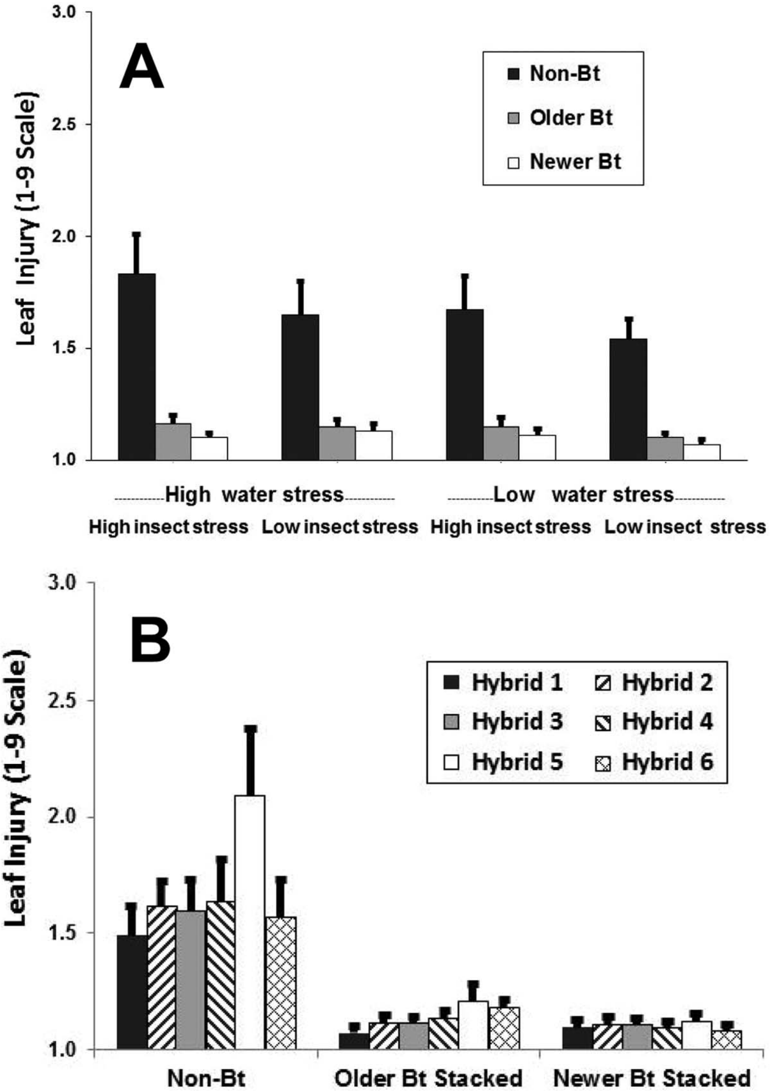

Fig. 2. Leaf injury (1–9 scale) means under high and low water stress and high and low insect stress for non-Bt hybrids, older Bt hybrids expressing Cry1Ab and Cry3Bb1, and newer Bt hybrids expressing Cry1A.105+Cry2Ab1 and Cry 3Bb2, averaged over six hybrid groups (A); and means of non-Bt, older Bt, and newer Bt versions of six hybrid groups, averaged over water stress and insect stress conditions (B). Lines are SEMs, 2012, Corpus Christi, TX. Leaf injury differences detected across the Bt transgene factor (B, $P < 0.05$), while no interactions were detected (see text for details).

larval penetration provided less discriminating but similar results. Consistent with leaf injury results, newer Bt hybrids had consistently low surface area of ear injury under both low and high insect stress, while non-Bt and older Bt hybrids had considerably greater ear injury especially under high insect stress (Bt transgene by insect stress interaction: $F = 59.4$; df = 2, 240; $P < 0.0001$; quadratic contrast across non-Bt and Bt hybrids within the interaction: $F = 14.0$; df = 1, 240; $P = 0.0002$; Fig. 3A). In contrast to higher leaf injury associated with high water stress (Fig. 1A), ear injury tended to be greatest under low water stress especially for the non-Bt hybrids. Ear injury decreased to intermediate levels on older Bt hybrids and was lowest on newer Bt hybrids (Bt transgene by water stress interaction: $F = 5.03$; df = 2, 240; $P = 0.007$; linear contrast across non-Bt and Bt hybrids within the interaction: $F = 8.53$; df = 1, 240; $P = 0.0038$; Fig. 3A). Also in contrast to uniform leaf injury across hybrid groups (Fig. 1B), ear injury was much higher in non-Bt and older Bt hybrids representing three of the six hybrid groups, while ear injury differences across newer Bt hybrids were seen to a lesser extent (Bt transgene by hybrid group interaction: $F = 9.06$; df = 10, 240; $P < 0.0001$; quadratic contrast across non-Bt and Bt hybrids within the interaction: $F = 24.8$; df = 1, 240; $P < 0.0001$; Fig. 3B). No other two-way or higher-order interactions were detected ($P > 0.10$). Measuring

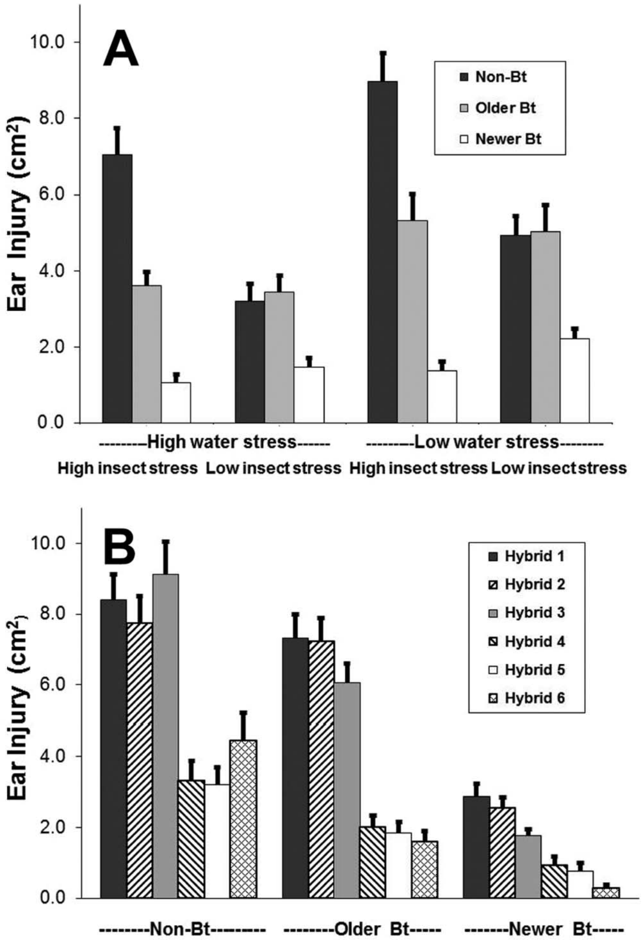

Fig. 3. Total ear injury (tip + kernels) means under high and low water stress and high and low insect stress for non-Bt hybrids, older Bt hybrids expressing Cry1Ab and Cry3Bb1, and newer Bt hybrids expressing Cry1A.105+Cry2Ab1 and Cry 3Bb2, averaged over six hybrid groups (A); and means of non-Bt, older Bt, and newer Bt versions of six hybrid groups, averaged over water stress and insect stress conditions (B). Lines are SEMs, 2011, Corpus Christi, TX. Bt transgene interactions with insect stress, water stress (A), and hybrid group (B) were detected ($P < 0.05$, see text for details).

depth of larval penetration, similar differences were observed for the Bt transgene by insect stress interaction ($F = 64.4$; df = 2, 240; $P < 0.0001$) and the Bt transgene by hybrid group interaction ($F = 5.84$; df = 10, 240; $P < 0.0001$), but the Bt transgene by water stress interaction was not significant ($P = 0.28$). For example, depth of larval penetration was 3.66 ± 0.18 and 1.93 ± 0.15 cm in non-Bt hybrids under high and low insect stress, respectively, while little ear injury occurred in newer Bt hybrids under high and low insect stress (0.73 ± 0.08 cm; full graphical data not shown).

In 2012, similar patterns to the previous year were seen in surface area of ear injury (Bt transgene by water stress interaction: $F = 4.5$; df = 2, 190; $P = 0.012$; Bt transgene by insect stress interaction: $F = 73.2$; df = 2, 190; $P < 0.0001$; Bt transgene by hybrid group interaction: $F = 9.50$; df = 10, 190; $P < 0.0001$). Ear injury was highest on non-Bt hybrids especially for low water stress conditions, intermediate on older Bt hybrids, and consistently low on newer Bt hybrids (linear contrast across non-Bt and Bt hybrids for the Bt transgene by water stress interaction: $F = 5.77$; df = 1, 190; $P = 0.0017$; Fig. 4A). The insecticide applications (i.e., low insect stress) at silking reduced ear injury of the non-Bt hybrids and older Bt hybrids, while newer Bt hybrids had low ear injury under high and low insect stress (quadratic contrast across non-Bt and Bt hybrids for the Bt transgene by insect stress interaction: $F = 63.6$; df = 1, 190; $P < 0.0001$; Fig. 4A). Ear injury was highest in non-Bt and older Bt hybrids representing three of the six hybrid groups, and some variation was seen in the newer Bt hybrids but to a lesser extent (Bt transgene by hybrid group interaction: $F = 37.0$; df = 1, 190; $P < 0.0001$; Fig. 4B). The hybrids showing high ear injury were the same hybrids experiencing high ear injury from corn earworm in 2011 (Fig. 3B). Interestingly, water stress, insect stress, and hybrid group interactions with the Bt transgene factor were seen when measuring total surface area ear injury

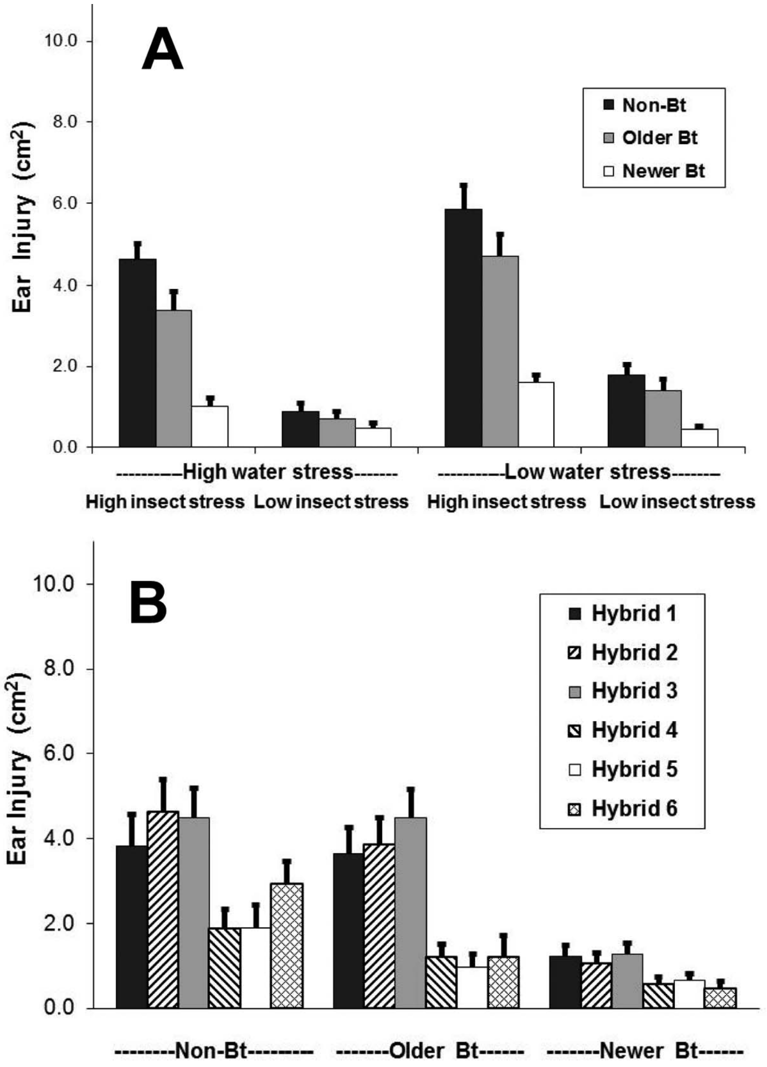

Fig. 4. Total ear injury (tip + kernels) means under high and low water stress and high and low insect stress for non-Bt hybrids, older Bt hybrids expressing Cry1Ab and Cry3Bb1, and newer Bt hybrids expressing Cry1A.105+Cry2Ab1 and Cry 3Bb2, averaged over six hybrid groups (A); and means of non-Bt, older Bt, and newer Bt versions of six hybrid groups, averaged over water stress and insect stress conditions (B). Lines are SEMs, 2012, Corpus Christi, TX. Bt transgene interactions with insect stress, water stress (A), and hybrid group (B) were detected ($P < 0.05$, see text for details).

(tip + kernel), while fewer interactions were observed when measuring kernel injury only (Bt transgene by insect stress interaction: $F = 18.8$; df = 2, 190; $P < 0.0001$) and depth of larval penetration (Bt transgene by insect stress interaction: $F = 32.3$; df = 2, 190; $P < 0.0001$, and Bt transgene by hybrid group interaction: $F = 2.84$; df = 10, 190; $P = 0.003$).

Yield Response and Association with Plant Injury. Despite no usable yield data from high water stress plots in 2012, yield differences and interactions were detected involving all available experimental factors. In 2011, newer and older Bt hybrids had generally higher yields than non-Bt hybrids, especially under low water stress when yield potential was high (Bt transgene by water stress interactions: $F = 5.03$; df = 2, 190; $P = 0.0007$; Fig. 5A). Lower yields were seen under high insect stress especially for non-Bt hybrids (Bt transgene by insect stress interaction: $F = 22.4$; df = 2, 190; $P < 0.0001$). Yield of the six hybrid groups within each Bt hybrid and non-Bt hybrids did not differ (no Bt transgene by hybrid group interaction was detected: $P = 0.23$), but three hybrid groups tended to have lower yields (hybrid group main effect: $F = 20.5$; df = 5, 100; $P < 0.0001$; Fig. 5B). In 2012, drought-related loss of the high water stress plots and yield depression in the low water stress plots led to fewer treatment differences (Fig. 6, note change in scale from Fig. 5). Yield increased steadily from non-Bt, older Bt, and newer Bt hybrids (Bt transgene main effect: $F = 20.8$; df = 2, 96; $P < 0.0001$; linear contrast:

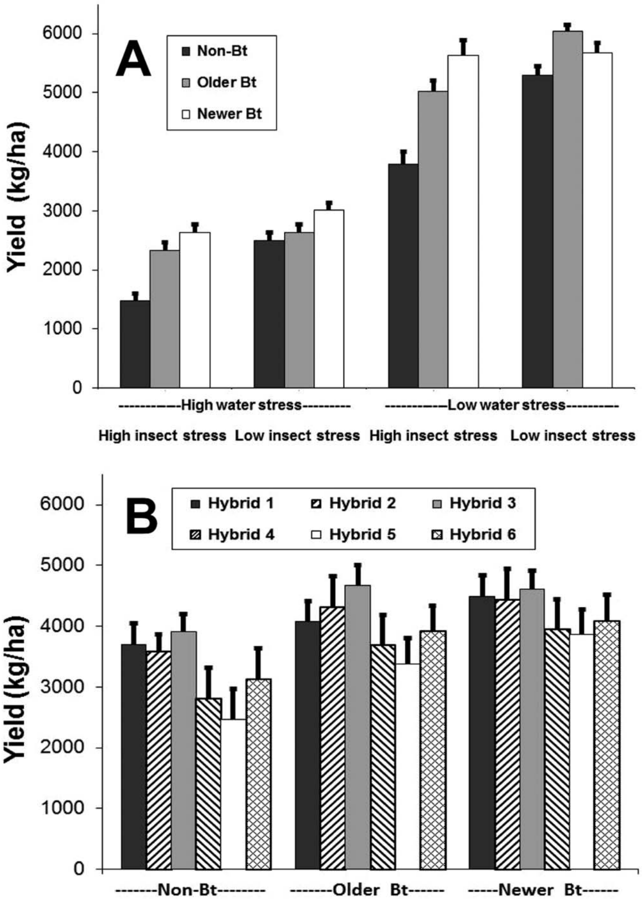

Fig. 5. Yield means under high and low water stress and high and low insect stress for non-Bt hybrids, older Bt hybrids expressing Cry1Ab and Cry3Bb1, and newer Bt hybrids expressing Cry1A.105+Cry2Ab1 and Cry 3Bb2, averaged over six hybrid groups (A); and means of non-Bt, older Bt, and newer Bt versions of six hybrid groups, averaged over water stress and insect stress conditions (B). Lines are SEMs, 2011, Corpus Christi, TX. Bt transgene interactions with insect and water stress (A) were detected ($P < 0.05$, see text for details).

$F = 39.5$; df = 1,096; $P < 0.0001$) under both high and low insect stress (i.e., no significant Bt transgene by insect stress interaction; Fig. 6A). Yield was higher in non-Bt and newer Bt hybrids representing three of the six hybrid groups, and yield was more variable for older Bt hybrids (Bt transgene by hybrid group interactions: $F = 3.08$; df = 10, 96; $P = 0.0002$; Fig. 6B). The hybrids of the higher yielding group were the same as those in 2011 (Fig. 5B), and unexpectedly these had higher ear injury as well (Figs. 3 and 4).

The incorporation of indicator variables in regressions of yield on leaf injury (guided by results from the leaf injury ANOVAs) also lent support to the strong influence of water stress on yield, with the lesser but interacting influence of leaf injury. The 2011 regression of yield on leaf injury was significant, when Bt transgene and water stress factors were inserted as indicator variables ($R^2 = 0.69$, $F = 132$; df = 7, 424; $P < 0.0001$). Separate regressions were estimated for high and low water stress conditions and common across non-Bt and Bt hybrids, because the water stress factor was relevant to the model ($F = 7.13$; df = 1, 424; $P = 0.008$) and the Bt transgene factor was not relevant ($P = 0.42$) based on model ($R^2 = 0.20$) and variable ($P \leq 0.15$) selection criteria. Yields of low water stress plots were about double that of high water stress plots, while increasing leaf injury was associated with further yield decline under both water stress conditions (Fig. 7A). Although yield differences among Bt and non-Bt hybrids were detected, the relevance of the Bt transgene factor on the regression of yield on leaf injury was low or possibly masked by the influence of water stress (Fig. 5). The other significant interacting factors detected in the ANOVAs (see previous section) were not incorporated into the regression models of yield on leaf injury in 2011 based on model and variable selection criteria. In 2012, leaf injury was very low in all treatments and no interactions were detected; therefore, regression models of yield on leaf injury were not considered.

The incorporation of indicator variables in regression models of yield on ear injury was significant in

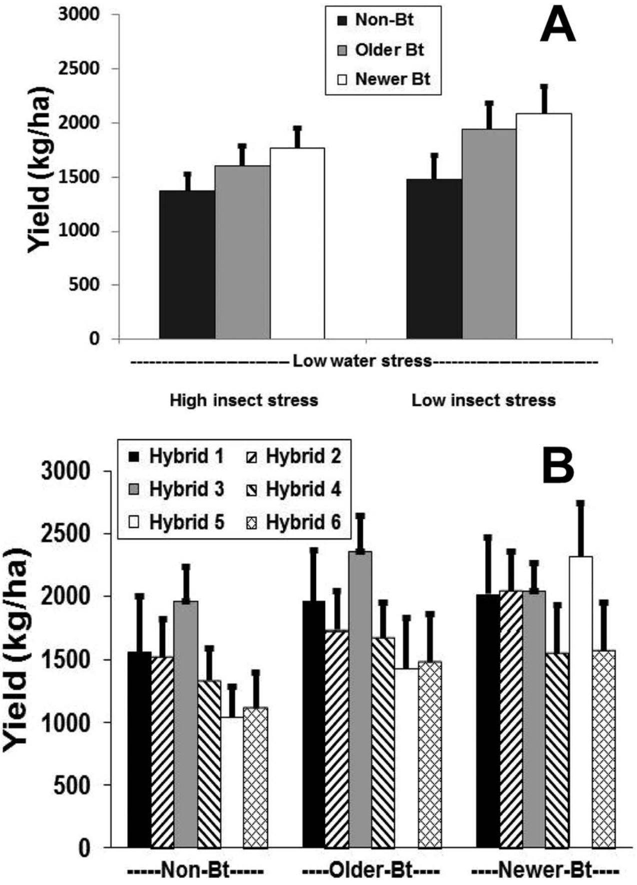

Fig. 6. Yield means under low water stress and high and low insect stress for non-Bt hybrids, older Bt hybrids expressing Cry1Ab and Cry3Bb1, and newer Bt hybrids expressing Cry1A.105+Cry2Ab1 and Cry 3Bb2, averaged over six hybrid groups (A); and means of non-Bt, older Bt, and newer Bt versions of six hybrid groups, averaged over insect stress conditions (B). Lines are SEMs, 2012, Corpus Christi, TX. Bt transgene interaction with hybrid group (B) was detected ($P < 0.05$, see text for details).

2011 ($R^2 = 0.68$, $F = 127$; df = 7, 424; $P < 0.0001$), and revealed no substantial association with water stress ($P = 0.24$) and a significant separation in regressions for non-Bt, older Bt, and newer Bt hybrids ($F = 7.37$; df = 2, 424; $P = 0.007$). Also when incorporating Bt transgene and the high- and low-yielding hybrid categories as indicator variables, the regression model of yield on ear injury was significant ($P < 0.0001$), the Bt transgene factor was relevant ($P = 0.02$), and the hybrid category did not meet the selection criteria ($R^2 = 0.12$). Therefore, separate regression lines were estimated for the non-Bt, older Bt, and newer Bt hybrids (Fig. 7B). Yield was higher for the Bt hybrids, and ear injury was less for the Bt hybrids, especially for the newer Bt hybrids (Fig. 3A, 7B). This result was consistent with the finding that ear injury was considerably reduced in the Bt hybrids while water stress had less effects (Fig. 3A). Interestingly, no significant yield change was observed for non-Bt hybrids across a wide range of ear injury observed (low slope and $R^2 = 0.02$ for the regression), but yield unexpectedly trended upward as ear injury increased for the Bt hybrids (Fig. 7B).

In 2012, the regression models of yield on ear injury and yield on kernel injury for low water stress plots and incorporating Bt transgene and insect stress factors as indicator variables were significant ($P < 0.05$) but did not meet the model selection criteria ($R^2 < 0.17$). Likewise, incorporating Bt transgene and the high- and low-yielding hybrid categories as indicator variables in the same regression models did not satisfy model selection criteria ($R^2 < 0.10$). Therefore, no regression models involving ear or kernel injury were estimated in 2012.

Discussion

From a stress perspective, high water stress led to increased leaf injury when substantial fall armyworm feeding pressure occurred in 2011 (Figs. 1 and 2), which was consistent with field observations that plants under water stress were more prone to insect injury and adverse yield effects (Haile 2000). A pulsed stress hypothesis has been proposed in which intermittent plant water stress, as seen in our experimentally manipulated water stress conditions, may posi-

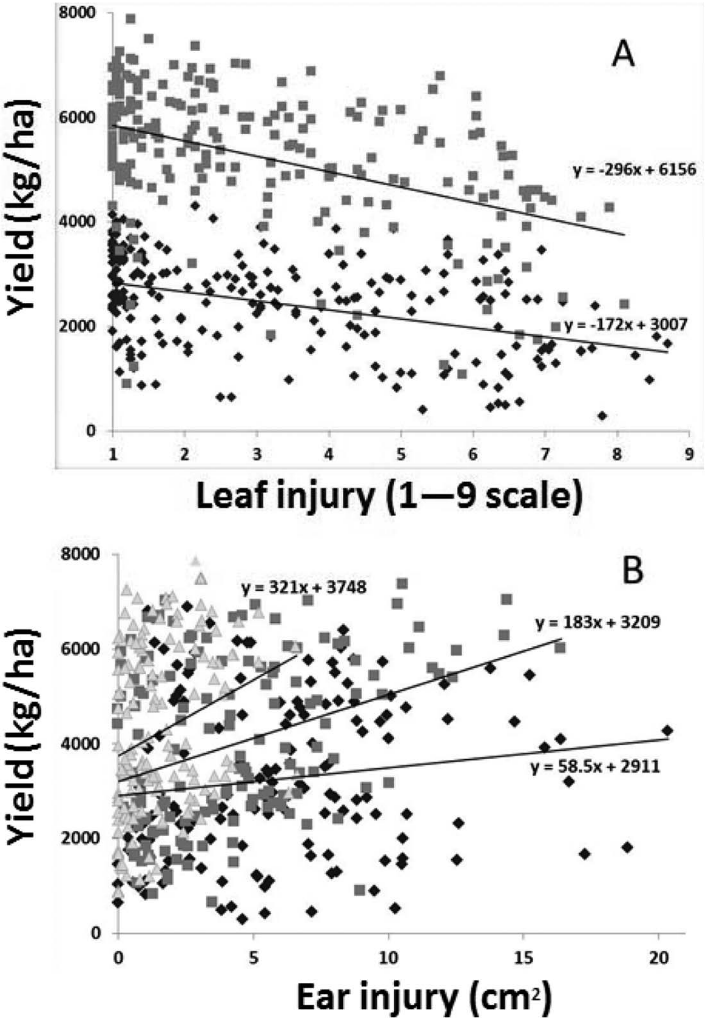

Fig. 7. Regressions of yield on plant injury variables using indicator variables to separate significant high water stress (diamond symbol, lower regression line and equation) and low water stress (square symbol, upper regression line and equation) conditions for leaf injury averaged across other factors (A, $R^2 = 0.69$, $P < 0.0001$, see text for details), and to separate significant non-Bt hybrids (diamond symbol, lower regression line and equation), older Bt hybrids expressing Cry1Ab and Cry3Bb1 (square symbol, middle regression line and equation), and newer Bt hybrids expressing Cry1A.105+Cry2Ab1 and Cry 3Bb2 (triangle symbol, upper regression line and equation) averaged across other factors (B, $R^2 = 0.68$, $P < 0.0001$, see text for details). 2011, Corpus Christi, TX.

tively affect insect performance leading to increased plant injury (Huberty and Denno 2004). Although proposed for sap-feeding insects, our leaf injury observations were consistent with this theory. In contrast, ear injury predominantly caused by corn earworm was greater in low water stress conditions, especially for the non-Bt hybrids (Figs. 3 and 4). Our late planted study facilitated high fall armyworm feeding pressure during vegetative growth, and high corn earworm feeding pressure during ear development. Corn earworm feeding within the ear husk differed considerably from the exposed fall armyworm feeding on leaves, which may result in differences in direct abiotic effects on the insects and indirect effects through differences in the water–nutrient balance of grain and leaves (see discussion in Haile [2000], Huberty and Denno [2004], and Showler [2013]). The more variable injury of non-Bt and older Bt hybrids expressing Cry1Ab and Cry3Bb1 would be expected to be more sensitive to the water and insect stress, as seen in our findings (Figs. 1–4).

From the perspective of plant injury and yield evaluation, leaf injury from fall armyworm and ear injury from corn earworm were lowest on more recent releases of Bt hybrids expressing Cry1A.105+Cry2Ab2 and Cry 3Bb1, compared with earlier Bt hybrids expressing Cry1Ab and Cry3Bb1 and non-Bt hybrids (Figs. 1–4). These results substantiated increased effectiveness of the newer Bt hybrids on fall armyworm and corn earworm reported by Siebert et al. (2012).

While a consistent reduction in leaf injury in the older and newer Bt hybrids across six hybrid backgrounds was observed (Figs. 1B and 2B), the mixed ear injury results of older Bt hybrids expressing Cry 1Ab were consistent with past observations on efficacy with older Bt transgenes (Buntin et al. 2004, Buntin 2008). Some variation in ear injury was observed in the newer Bt hybrids as well (Figs. 3B and 4B), in contrast to the more consistent performance reported by Siebert et al. (2012).

Plant injury effects on yield as influenced by Bt transgenes were important, but Bt transgenes played a more variable and lesser role when interacting with water stress to affect yield (Figs. 5–7). For example, yield sensitivity to water stress in 2011 was high (Fig. 5A) and was less influenced by the extent of leaf injury (as high as 8.7 on a 1–9 scale) from fall armyworm (Fig. 7A). Any influence of ear injury on yield was even more variable: it was difficult to explain no significant yield change for the non-Bt hybrids and yield trending upward for the Bt hybrids as ear injury increased (Fig. 5B). These interactions of corn genetics (i.e., Bt transgenes and hybrid background) and stress (i.e., water and insect stress) may be partly responsible for at times conflicting reports of yield response and leaf and ear injury caused by fall armyworm and corn earworm when evaluating Bt hybrids with older Bt transgene packages (Horner et al. 2003, Buntin et al. 2004, Buntin 2008, Chilcutt et al. 2006). Other associated stresses (e.g., disease stress caused by the potential of these insects to introduce ear molds; Dowd 2001) may further complicate yield response, including that seen in our study (G.N.O., personal observation).

The contrasting results of in-season plant injury measurements and yield provided guidance for future experiment work. Water stress as an experimental factor affected plant injury and yield, and was particularly relevant given common water stress and ongoing drought episodes experienced in the southern United States and elsewhere (Haile 2000, Baumhardt and Salinas-Garcia 2006). Improved soil water measurements and adding physiological plant responses (Kebede et al. 2012) would allow quantification of water stress beyond our high and low water stress categories and better prevent harvest complications experienced in this study. Also, different feeding habits of leaf injury and ear injury caused by two species may be confounding interactions with water stress and yield response. Therefore, experimental manipulation to consider each form of injury in isolation would be beneficial, as well as measuring insect growth and fitness indicators (Horner et al. 2003, Showler 2013). We have shown the relevance of water stress in considering the potential yield effects of the newer Bt transgene packages, but monetary cost in using the Bt transgene technology was not considered. In our experiment, the detected yield gains were primarily associated with alleviating water stress, and to a lesser extent insect control from using Bt hybrids. To address the economic aspect of deploying Bt transgenes for control of these insects, accounting of costs of using hybrids with Bt transgenes and value of yield gains from insect control will be essential. This should be done across a wide range of yield potential associated with water stress relevant to corn production regions of interest, such as in the southern United States.

These results exemplify the interplay of water and insect stress with plant injury and yield, their interactions with Bt transgene packages, and the importance of these interactions in considering Bt transgene use and adoption strategies. While newer Bt hybrids expressing Cry1A.105+Cry2Ab2 and Cry 3Bb1 had consistent low leaf injury and high yield and low but less consistent ear injury across six hybrid backgrounds, water stress was a key factor that influenced yield. Overall, strategies of use of Bt hybrids should consider the major influence of water stress along with its interaction with Bt transgenes in affecting plant injury and yield when considering their use in corn production regions where water stress is common.

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