Goss’s Wilt Incidence in Sweet Corn Is Independent of Transgenic Traits and Glyphosate

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Abstract. Recently, claims have been made that the use of glyphosate and transgenic crop traits increases plant susceptibility to pathogens. Transgenic traits used widely for years in dent corn are now available in commercial sweet corn cultivars, specifically, the combination of glyphosate resistance (GR) and Lepidoptera control (Bt). The objective was to assess the interactions of the GR+Bt trait, glyphosate, and Goss’s wilt on sweet corn. Nine treatments were tested under weed-free conditions at two sites in 2013 and 2014. Treatments included two isogenic cultivars differing only in the presence or absence of GR+Bt, with and without postemergence application of glyphosate, and inoculation with the causal agent of Goss’s wilt (Clavibacter michiganensis ssp. nebraskensis) before glyphosate application, after glyphosate application, or no inoculation. Results failed to show glyphosate or the GR+Bt trait influenced sweet corn susceptibility to Goss's wilt. The only factor affecting Goss’s wilt incidence was whether plants were inoculated with C. michiganensis ssp. nebraskensis. In the absence of glyphosate application, yet under weed-free conditions, several yield traits were higher in sweet corn with the GR+Bt trait. Results showed that the GR transgene confers the same level of tolerance to glyphosate in sweet corn as observed previously in dent corn. If true, recent claims about glyphosate and transgenic traits increasing plant disease would be of major concern in sweet corn; however, no relationships were found between the GR+Bt trait and/or glyphosate to Goss’s wilt incidence in sweet corn.

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Weed and insect pest management in agronomic crops has changed dramatically since commercialization of transgenic glyphosate [N-(phosphonomethyl)glycine]-resistant (GR) cultivars in the 1990s. The ability to use glyphosate, a nonselective herbicide, in previously sensitive crops made weed management in GR crops simpler and less expensive (Duke and Powles, 2008). In dent corn (Zea mays L.), developers of GR cultivars also engineered the plants to produce toxins effective against Lepidoptera larvae, commonly known as the Bt trait. Largely, because the GR+Bt combination appeared to simplify weed and insect pest management and at an economical advantage to pest management in non-Bt glyphosate-sensitive (GS) corn, transgenic cultivars were rapidly adopted in the United States (Green, 2012). The U.S. growers adopted GR+Bt dent corn on more than 80% of the corn area within 10 years of introduction (James, 2011).

Using the same transgenic traits employed in dent corn, GR+Bt sweet corn cultivars were commercialized in 2011 (Seminis, 2015). Although adoption rates of GR+Bt sweet corn have yet to be published, many growers in the Midwest United States eagerly waited to embrace GR+Bt technology in sweet corn (Anonymous, 2015).

In recent years, claims have been made that glyphosate use and the GR trait increases plant susceptibility to pathogens. Johal and Huber (2009) speculated a hypothetical glyphosate toxicity in GR crops would enhance their vulnerability to fungal diseases following glyphosate application. Others have proposed increased disease susceptibility due to an adverse effect of glyphosate on plant mineral nutrition (Yamada et al., 2009). Although the preponderance of evidence indicates that disease susceptibility in GR crops is driven by the inherent level of disease resistance in the host plant and not by the presence of the GR gene or treatment with glyphosate (Duke et al., 2012), the vast majority of such research has been conducted in dent corn and soybean [Glycine max (L.) Merr.]. Susceptibility to a wide range of diseases has been problematic in sweet corn since the mid-19th century (Huelson, 1954). While improvement in tolerance to specific diseases has been observed (e.g., common rust), sweet corn germplasm remains susceptible to several other prevalent diseases because of the greater importance to breeders of improving yield and eating-quality traits (Patak1 et al., 2011).

One of the diseases many sweet corn and dent corn germplasm lines are susceptible to is Goss’s bacterial wilt and blight (caused by C. michiganensis ssp. nebraskensis). Goss’s wilt was first identified in Nebraska in 1969, Iowa in 1971, and Kansas in 1972 (Wysong et al., 1973). Within 12 years of the first discovery in Nebraska, Goss’s wilt was observed in additional states across the Plains and the Midwest, which included Colorado, Illinois, and South Dakota (Wysong et al., 1981). Incidence of Goss’s wilt in midwestern states, such as Illinois, continued to be very low until the late 2000s and early 2010s. During this period, Goss’s wilt was reported for the first time in Indiana (Ruhl et al., 2009), Minnesota (Malvick et al., 2010), and North Dakota (Friskop et al., 2014). During this time, Goss’s wilt also began to reemerge in the central High Plains in the United States (Jackson et al., 2007). Two phases of the disease can occur, a leaf blight and a systemic wilt. Both phases of the disease can occur in a field, but the systemic wilt phase is the most destructive (Suparyono and Pataky, 1989). Infections by the causal bacteria occur through wounds in plant tissue caused by injury such as hail or sandblasting (Claffin, 1999). The systemic wilt phase...
occurs when the bacteria reaches the xylem tissue where they spread, limiting water movement. Symptoms of leaf blight can appear as large lesions with wavy margins and irregular, dark spots within the lesions (typically referred to as “freckles”). Yield of sweet corn (both ear weight and number of ears) can be reduced greatly, especially when infections occur in the early stages of plant development (Suparyono and Pataky, 1989).

Although scientific evidence of glyphosate applications having an effect on Goss’s wilt has not been published (Duke et al., 2012), Langemeyer (2012) reported that a significant, positive correlation was observed between glyphosate application and detection of C. michiganensis ssp. nebraskensis in corn leaf samples. In addition, Huber (2011) speculated that surfactants applied with herbicides could change the host and facilitate penetration by Clavibacter.

The objective of the present study was to assess the interactions of the GR+Bt trait, glyphosate, and Goss’s wilt on sweet corn.

Materials and Methods

A single protocol was used to conduct field experiments in 2013 and 2014 near Urbana, IL. Experiments were conducted at two locations at the University of Illinois Crop Sciences Research and Education Center near Champaign–Urbana, IL; one at the Fruit Research Farm and the other at the Vegetable Crop Research Farm. Different fields were used each year. The soil at the Fruit Farm was a Dana silt loam (fine-silty, mixed, superactive, mesic Oxyaquic Argudolls) averaging 6.9% organic matter and a pH of 6.4. The soil at the Vegetable Crop Farm was a Drummer silty clay loam (fine-silty, mixed, superactive, mesic Typic Endoaquolls) averaging 3.3% organic matter and a pH of 6.0. Within two weeks of planting, 135 kg N/ha was applied as urea and immediately incorporated with a field cultivator. Sweet corn was planted on 76-cm rows at 69,000 plants/ha on 16 May 2013 (Vegetable Crop Farm), 17 May 2013 (Fruit Farm), and 21 May 2014 (both sites). As a preventative measure for control of corn rootworms (Diabrotica spp.) in all plots, tefluthrin [(2,3,5,6-tetrafluoro-4-methylphenyl) methyl] (1R,3R)-rel-[(12)-2-chloro-3,3,3-trifluoro-1-propenyl]-2,2-dimethylcycloprop-2-ene carboxylate) was applied in a t-band at planting. The entire site was maintained weed free using a preemergence application of simazine [2-chloro-N(2-ethyl-6-methylphenyl)-N-(15-2-methoxy-1-methylethyl) acetamide] with atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) and weekly hand weeding. Any sign of drought stress (e.g., leaf rolling) was alleviated with supplemental sprinkler irrigation, which occurred twice in the 2013 Vegetable Crop Farm field.

Experimental approach. The experimental design was a randomized complete block with four replications. Nine treatments (Table 1) were assigned to experimental units of four crop rows spaced 76 cm apart and measuring 9.2 m in length. Cultivars were isogenic, differing only in the presence (‘Passion II’) or absence (‘Passion’) of two transgenes; one transgene (cp4 epsp) conferring resistance to glyphosate and another transgene (cry3 Bb1) conferring resistance to corn rootworms (Monsanto, St. Louis, MO). In treatments receiving glyphosate, the formulated product was applied using a compressed air backpack sprayer at a rate of 1.68 kg a.e./ha in 187 L·ha⁻¹ of spray volume when the crop had 4 to 5 visible collars (4V to 5V). The plots that included inoculation with C. michiganensis ssp. michiganensis, the pinprick inoculation method was used (Blanco et al., 1977; Pataky, 1985). This method involved growing C. michiganensis ssp. nebraskensis (originally isolated from a corn leaf collected in Champaign County, IL, in 2011) in nutrient broth (Becton, Dickinson and Company, Franklin Lakes, NJ) on a shaker table for 2 d at room temperature (23–25 °C) under constant light. Bacterial suspensions were adjusted to 1 × 10⁷ cfu/mL using a 0.1 M NaCl solution before inoculations. This inoculum was used to inoculate early planted ‘Jubilee’ sweet corn grown in a nearby field to increase inocula. Ten symptomatic leaves collected from the ‘Jubilee’ disease nursery were cut into smaller pieces (≤5 cm²), placed into a commercial blender (Model 38BL52; Waring Products, New Hartford, CT) containing 3.8 L of a 0.1 NaCl solution, and blended for 30 s. The blended leaf suspension was then strained through a kitchen strainer to remove larger pieces of leaf tissue. This suspension was then used to inoculate plants using the pinprick method (Blanco et al., 1977; Pataky, 1985). Plants were inoculated twice within a 24-h period in appropriate plots, either before or after glyphosate application.

Data collection. Incidence (%) of plants with Goss’s wilt symptoms was assessed 22 and 25 d after glyphosate application in 2013 and 2014, respectively. Specifically, the number of plants each with foliar or systemic symptoms that exhibited Goss’s wilt symptoms were counted by plot. Each experiment was harvested 18 d after midsilk. Harvest dates ranged from July 29 to July 31. Marketable ears, measuring ≥4.5 cm in diameter, were hand harvested over the center 6.1 m of the middle two rows of each plot. Ear number and mass were recorded. Ten harvested ears were randomly selected from each plot, hand husked, and weighed. Fresh kernels were cut from the cob with an industry-grade corn cutter (A&K Development, Eugene, OR). Cob mass was recorded. Kernel mass was calculated as the difference in husked mass and cob mass. Recovery was calculated as the percentage of kernel mass represented in the mass of the ear sample before husking. Cut kernel moisture was determined gravimetrically using a drying oven set to 40 °C for 72 h.

Data analysis. Before analysis, data were examined with Levene’s test for homogeneity of variances (Ott and Longnecker, 2001). Variances were found to be homogeneous and met analysis of variance (ANOVA) assumptions of normality. Response variables were analyzed separately by ANOVA using the Proc Mixed procedure of SAS, version 9.3 (SAS Institute, 2010). Years and sites were considered random effects. Cultivar, herbicide, and inoculation were considered fixed effects. Since the experimental design was unbalanced (i.e., lacking the treatment of a GR+Bt cultivar treated with glyphosate, a lethal combination), two separate two-factor ANOVAs were conducted. Specifically, all plots not receiving a glyphosate application (i.e., herbicide free) were analyzed with an ANOVA model containing cultivar, inoculation, and their interaction as factors. A separate model with factors of herbicide, inoculation, and their interaction were used to examine all plots with GR+Bt sweet corn. Since disease incidence was zero in noninoculated plots, the inoculation factor of both ANOVA models had two levels (as opposed to three) for disease incidence: C. michiganensis ssp. nebraskensis inoculation before and after glyphosate application. All effects were declared significant at $P < 0.05$.

Results

Disease incidence. About one-half of the inoculated plants showed Goss’s wilt symptoms 3 to 4 weeks after inoculation. Symptoms included leaf lesions that were expanding from the initial site of inoculation. Leaf lesions observed were typical of Goss’s wilt, and were light green/yellow to gray in color with wavy margins and dark “freckles” inside of the lesions. Goss’s wilt severity was limited to leaf lesions, with few plants (<3%) showing symptoms of systemic infection (data not shown).

Timing of C. michiganensis ssp. nebrakensis inoculation, either before or after glyphosate application, had no effect on Goss’s wilt incidence. About one-half of the inoculated plants showed Goss’s wilt symptoms 3 to 4 weeks after inoculation. Symptoms included leaf lesions that were expanding from the initial site of inoculation. Leaf lesions observed were typical of Goss’s wilt, and were light green/yellow to gray in color with wavy margins and dark “freckles” inside of the lesions. Goss’s wilt severity was limited to leaf lesions, with few plants (<3%) showing symptoms of systemic infection (data not shown).

Table 1. Nine treatments tested in sweet corn grown in two sites near Urbana, IL, in 2013 and 2014.

<table>
<thead>
<tr>
<th>Cultivar*</th>
<th>Herbicide*</th>
<th>Inoculation*</th>
</tr>
</thead>
<tbody>
<tr>
<td>GR+Bt</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>GR+Bt</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>GS</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>GR+Bt</td>
<td>+</td>
<td>Before herbicide application</td>
</tr>
<tr>
<td>GR+Bt</td>
<td>–</td>
<td>Before herbicide application</td>
</tr>
<tr>
<td>GS</td>
<td>+</td>
<td>Before herbicide application</td>
</tr>
<tr>
<td>GR+Bt</td>
<td>–</td>
<td>+ After herbicide application</td>
</tr>
<tr>
<td>GR+Bt</td>
<td>+</td>
<td>+ After herbicide application</td>
</tr>
<tr>
<td>GS</td>
<td>–</td>
<td>+ After herbicide application</td>
</tr>
<tr>
<td>GR+Bt</td>
<td>+</td>
<td>+ After herbicide application</td>
</tr>
<tr>
<td>GS</td>
<td>–</td>
<td>+ After herbicide application</td>
</tr>
</tbody>
</table>

*GR-Bt was a glyphosate-resistant Bt cultivar; GS was a nontransgenic, glyphosate-sensitive isoline. With (+) or without (–) glyphosate applied at 1.68 kg a.e./ha to four- and five-collar corn.

With (+) or without (–) Goss’s wilt inoculation on the dates of 12 June 2013 and 16 June 2014 for “before herbicide application,” and 14 June 2013 and 19 June 2014 for “after herbicide application.”
Table 2. Significance of sweet corn cultivar (C), inoculation (I), herbicide (H), and relevant two-way interactions.

<table>
<thead>
<tr>
<th>Dataset</th>
<th>Factor</th>
<th>Ear number</th>
<th>Ear mass</th>
<th>Kernel mass</th>
<th>P value</th>
<th>Recovery</th>
<th>Kernel moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herbicide free</td>
<td>C</td>
<td>0.004</td>
<td>0.056</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.010</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>0.054</td>
<td>0.267</td>
<td>0.242</td>
<td>0.768</td>
<td>0.755</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C*I</td>
<td>0.611</td>
<td>0.643</td>
<td>0.975</td>
<td>0.419</td>
<td>0.656</td>
<td></td>
</tr>
<tr>
<td>GR+Bt</td>
<td>H</td>
<td>0.002</td>
<td>&lt;0.001</td>
<td>0.014</td>
<td>0.379</td>
<td>0.735</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>0.170</td>
<td>0.696</td>
<td>0.439</td>
<td>0.149</td>
<td>0.285</td>
<td></td>
</tr>
<tr>
<td></td>
<td>H*I</td>
<td>0.563</td>
<td>0.582</td>
<td>0.485</td>
<td>0.802</td>
<td>0.657</td>
<td></td>
</tr>
</tbody>
</table>

1Herbicide-free dataset includes plots not receiving glyphosate; GR+Bt dataset includes plots of the glyphosate-resistant Bt cultivar.

Table 3. Means of crop yield traits.

<table>
<thead>
<tr>
<th>Dataset</th>
<th>Cultivar</th>
<th>Ear number (boxes/ha)</th>
<th>Ear mass (t/ha⁻¹)</th>
<th>Kernel mass (t/ha⁻¹)</th>
<th>Recovery (%)</th>
<th>Kernel moisture (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herbicide-free</td>
<td>GR+Bt</td>
<td>1,360 a</td>
<td>18.9 a</td>
<td>5.78 a</td>
<td>30.4 a</td>
<td>82.4 b</td>
</tr>
<tr>
<td></td>
<td>GS</td>
<td>1,270 b</td>
<td>18.0 a</td>
<td>5.03 b</td>
<td>27.6 b</td>
<td>83.0 a</td>
</tr>
<tr>
<td>GR+Bt</td>
<td>+</td>
<td>1,460 a</td>
<td>20.8 a</td>
<td>6.26 a</td>
<td>29.9 a</td>
<td>82.5 a</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>1,360 b</td>
<td>18.9 b</td>
<td>5.78 b</td>
<td>30.4 a</td>
<td>82.4 a</td>
</tr>
</tbody>
</table>

*Means followed by the same letter within a dataset and response variable are not significantly different (P > 0.05).

incidence (P ≥ 0.602). Moreover, cultivar, herbicide, and their interactions with inoculation timing did not influence disease incidence (P ≥ 0.324). For example, disease incidence averaged 50.7% in both GR+Bt and GS plots.

Transgenes and C. michiganensis ssp. nebraskensis inoculation. On glyphosate-free treatments, C. michiganensis ssp. nebraskensis inoculation appeared to have little, if any, effect on sweet corn (Table 2). With the exception of a marginally insignificant test statistic for ear number (P = 0.054), C. michiganensis ssp. nebraskensis inoculation did not influence yield traits (P ≥ 0.242). Moreover, cultivars by inoculation interactions were insignificant (P ≥ 0.419) for all yield traits.

In contrast, crop yield was affected by cultivar. Several measures of yield of the GR+Bt cultivar were 5% to 15% higher than the GS cultivar (Table 3). Kernel mass was 5.78 and 5.03 t/ha⁻¹ for cultivars GR+Bt and GS, respectively. Kernel moisture at the time of harvest was 0.6% lower in the GR+Bt cultivar.

Glyphosate and C. michiganensis ssp. nebraskensis inoculation. Of the GR+Bt treatments, C. michiganensis ssp. nebraskensis inoculation had no effect on sweet corn yield (P ≥ 0.170; Table 2), nor were glyphosate by inoculation interactions significant (P ≥ 0.485).

In contrast, glyphosate application affected ear number, ear mass, and kernel mass (P ≤ 0.014; Table 2). Ear number, ear mass, and kernel mass averaged 8% higher in glyphosate-treated plots, relative to untreated plots (Table 3). Recovery and kernel moisture were unaffected by cultivar.

**Discussion**

Results failed to show glyphosate or the GR+Bt trait influenced sweet corn susceptibility to Goss’s wilt in the cultivars tested. In herbicide-free plots inoculated with C. michiganensis ssp. nebraskensis, Goss’s wilt incidence was not influenced by cultivar, inoculation timing, nor their interaction. In GR+Bt plots inoculated with C. michiganensis ssp. nebraskensis, Goss’s wilt incidence was not influenced by glyphosate use, inoculation timing, nor their interaction. The only factor affecting Goss’s wilt incidence was whether plants were inoculated with C. michiganensis ssp. nebraskensis. Inoculated plants averaged 50% disease incidence, whereas noninoculated plants were asymptomatic.

In the absence of glyphosate application, yet under weed-free conditions, ear number, kernel mass, and recovery were higher in sweet corn with the GR+Bt trait. Stalk- and ear-feeding insects were not observed in either isolate. Higher kernel moisture observed in the GS isolate suggested development of that cultivar was delayed with respect to the GR+Bt cultivar, since kernel moisture decreases with maturity for endosperm mutants used widely in sweet corn (Azanza et al., 1996). Subtle developmental delays greatly reduce sweet corn yield. Williams (2010) showed a 2-d delay in silk emergence due to weed interference decreased marketable ear number 37%. Factors other than weed interference were involved in the present work, as all plots were kept weed free. After crop emergence, hand weeding was used to keep plots weed free, which was used extensively in all GS plots but less in GR+Bt plots. Perhaps, extra foot traffic led to some soil compaction in GS plots, thereby impacting root growth, nutrient uptake, and ultimately crop yield.

**Literature Cited**


