Conventional Screening Overlooks Resistance Sources: Rootworm Damage of Diverse Inbred Lines and Their B73 Hybrids Is Unrelated

SHERRY A. FLINT-GARCIA, KENTON E. DASHIELL, DEIRDRE A. PRISCHMANN, MARTIN O. BOHN, AND BRUCE E. HIBBARD

J. Econ. Entomol. 102(3): 1317–1324 (2009)

ABSTRACT The western corn rootworm, *Diabrotica virgifera virgifera* (LeConte), is a major pest of maize, *Zea mays* L., in the United States and Europe, and it is likely to increase in importance as a trend toward increased nonrotated maize favors larger rootworm populations. Options for rootworm management in nonrotated maize in Europe and in nontransgenic “refuge” areas in countries that permit transgenic maize are limited to insecticides. Development of additional options for growers would be helpful. Screening maize germplasm (e.g., landraces, populations, inbreds) for native resistance to western corn rootworm is labor-intensive and is usually conducted on unfinished germplasm and not on hybrid materials. However, we have recently observed that topcrossed (hybrid) materials tend to have reduced western corn rootworm damage. To formally test whether rootworm damage to inbreds and associated hybrids were correlated, we evaluated 25 diverse inbred lines and their B73 hybrids for western corn rootworm damage in seven environments. Overall, hybrids had significantly less damage than inbreds, but unfortunately, the correlation between inbreds and hybrids was not significant. These findings have important implications regarding screening germplasm for western corn rootworm resistance, namely, that inbred materials and perhaps populations should be topcrossed to form hybrid materials before screening for western corn rootworm damage to ensure that valuable sources of resistance to western corn rootworm are not missed during the screening process.

KEY WORDS *Diabrotica virgifera virgifera*, western corn rootworm, heterosis, native plant resistance

The western corn rootworm, *Diabrotica virgifera virgifera* (LeConte), is the most important pest of maize, *Zea mays* L., in the United States, and it is rapidly growing in importance in Europe (Gray et al. 2009). It is primarily a pest in its larval stages; the larvae feed on the roots of maize. Maize root damage can disrupt water relations (Riedell 1990), reduce nutrient uptake (Kahler et al. 1985a), reduce photosynthetic rate (Godfrey et al. 1993), and reduce plant stability (i.e., cause root lodging; Spike and Tollefson 1989). Losses from adult feeding on silk before pollination also can be important in some situations, especially in seed production fields (Culy et al. 1992). Economic losses are estimated to be $650 million to $1 billion annually in yield reduction and control costs in the United States (Gray 2000).

Management strategies for the western corn rootworm in the United States include crop rotation, application of insecticides, use of transgenic varieties with resistance to western corn rootworm larval feeding, or a combination. However, the insect has proven adaptable and has modified its oviposition behavior to become resistant to crop rotation (Levine et al. 2002) and developed resistance to insecticides targeted for larval (Ball and Weekman 1962) and adult control (Meinke et al. 1998). Currently, there are several transgenic events commercially available for rootworm management that express insecticidal proteins derived from the bacteria *Bacillus thuringiensis* Berliner (Bt). Although different hybrids expressing the same transgenic event can provide different control (Gray et al. 2007), overall transgenic corn targeted toward corn rootworm management has been effective as indicated by its increasing market share. Resistance to Bt maize has been selected for under laboratory conditions (Lefko et al. 2008, Meihls et al. 2008) and experimentally documented under field conditions (Meihls et al. 2008). Although some types of granular soil insecticides applied in a band over the row or in-furrow have remained effective for >40 yr,
perhaps because of survival outside the treated root zone (Levine and Olumi-Sadeghi 1991), the long-term durability of Bt maize targeted toward the western corn rootworm remains unknown.

The Environmental Protection Agency (EPA) has mandated that all registrants submit an insect resistance management (IRM) plan before registration of any Bt crop. The current IRM plan for Bt crops targeting rootworms require that 20% non-Bt “refuge” be planted within or adjacent to the Bt field where the pests do not encounter Bt toxin. It is expected that a large number of susceptible rootworms emerging from the refuge will mate with any resistant individuals emerging from the Bt field to produce heterozygous susceptible offspring and thus delay the evolution of pest resistance. Currently, rootworm management on refuge fields and nonrotated maize in Europe is limited to insecticides.

One potential alternative to transgenic resistance and insecticides for western corn rootworm management is native plant resistance. Several public and commercial programs have screened germplasm to identify and develop germplasm with resistance (Moeser and Hibbard 2005, Tollefson 2007), and promising sources of resistance have been identified (Branson et al. 1983; Kahler et al. 1985b; Assabgui et al. 1995; Hibbard et al. 1999, 2007). Although some overall progress has been made in terms of tolerance to western corn rootworm larval feeding from the 1960s to the 1980s (Riedell and Evenson 1993), no hybrids claiming native resistance to western corn rootworm larval feeding are currently commercially available. Why? Perhaps previous workers in the area of corn rootworm native plant resistance were evaluating germplasm in the wrong form. Typically in rootworm breeding programs, screening has been done using inbred lines, populations, or landraces, but not as topcrosses (i.e., hybrid crosses). However, when a source of native western corn rootworm resistance was crossed to elite inbreds, the resulting topcrosses were less damaged than the resistant parent, even when the elite line used as the topcross parent was susceptible to rootworm feeding (Hibbard et al. 2007). Given that farmers grow hybrids, not inbreds or populations, breeders need to understand how inbreds perform in hybrid combinations.

The objective of this study was to formally examine the relationship between inbred and hybrid performance in terms of feeding injury from western corn rootworm larvae. In other words if a resistant inbred is identified, will its hybrid crosses also be resistant?

Materials and Methods

Plant Materials. Twenty-six maize inbred lines picked to represent the range of genetic diversity present in publicly available maize breeding germplasm (Liu et al. 2003, Flint-Garcia et al. 2005) were chosen for this study: B73, B97, CML103, CML228, CML247, CML277, CML322, CML333, CML 52, CML69, HP301, IL14H, Ki11, Ki3, Ky21, M162W, M37W, Mo17, Mo18W, MS71, NC350, NC358, Oh43, Oh7B, P39, and Tx303. The inbred B73 was used as a female to produce hybrid seed of the 25 remaining lines (note that B73 was also one of the 26 inbred lines evaluated and is being sequenced by the maize genome project).

Experimental Design and Phenotypic Evaluation. Genotypes were arranged in a randomized complete block design at seven environments with three replications in each environment. Environments included the Bradford Research and Extension Center near Columbia, MO (one environment in 2006, and two environments in 2007); Poultry Farm near Columbia, MO (2007); a site near Marshall, MO (2007); a site near Brookings, SD (2007); and a site at the University of Illinois Research and Education Center in Urbana, IL (2007). The two Bradford Farm environments in 2007 were planted 5 d apart in different fields. Entries evaluated included the 26 inbred lines and the 25 B73-hybrids. A susceptible control line (B37×H54) was included in all environments. In 2007, B37×H54 treated with a soil insecticide (telithrin at the recommended rate of 113 g/305 m of row) and DKC 60–12 (transgenic rootworm-resistance conferred by the MON863 event expressing the Cry3Bb1 protein) were added as resistant controls.

In all Missouri environments, nine kernels were planted in 1.8-m-long single row plots. In Brookings, eight kernels were planted in 1.8-m-long single row plots, and in Urbana 15 kernels were planted in 4.5-m-long single row plots. Methods of western corn rootworm infestation included artificial infestation at the Brookings, Poultry Farm, and Bradford Farm environments, and a trap crop for natural infestation at the Poultry Farm, Marshall, and Urbana. The Missouri trap crops consisted of mixed maturity maize (90–125 d) planted 1 wk later than the plots. The Urbana trap crop consisted of a 110 d maturity maize hybrids mixed with pumpkin seed (1 kg per acre, ‘Sugar and Pie’, 90 d) planted 2 wk after all other western corn rootworm experiments and screening nurseries were established in 2006. Poultry Farm also was artificially infested with a reduced rate of eggs to augment the egg load at this environment because natural egg densities were traditionally not as high at Poultry Farm as at the Urbana or Marshall environments. Western corn rootworm eggs were provided by the USDA-ARS North Central Agricultural Research Laboratory, Brookings, SD. For artificial infestation in Missouri, eggs were suspended in 0.15% agar solution (Palmer et al. 1977) and were mechanically infested (Moellenbeck et al. 1994) at the V2 stage of plant development with ≈1,000 eggs per 30.5 cm of row at the Bradford environments, and 500 eggs per 30.5 cm of row at Poultry Farm. At the Brookings environment, eggs were mechanically infested at a rate of 1,100 eggs per 30.5 cm by using the technique of Sutter and Branson (1980) immediately before hand planting.

Before root excavation, plot information was affixed to five plants per plot in Urbana and four roots per plot in all other environments. When a sufficient number of plants per plot were present, the sampled plants were not consecutive, nor end plants in the plot. Roots were dug after most western corn rootworm feeding.
was completed. This was determined by monitoring growing degree-days (600 growing degree-days according to Fisher et al. [1990]) and by digging up plants from infested, nonexperimental check plots. Excavation was accomplished using a specially designed, tractor-mounted implement (Praiswater et al. 1997) at all Missouri environments, spades in Urbana, and potato forks in Brookings. Roots were soaked, washed, and rated for damage using the 0–3 scale described in Oleson et al. (2005).

Data Analysis. Because of poor seed germination or growth, ≈3% of the plots were not scorable across all environments. To cope with missing data, the PROC MIXED procedure of SAS (SAS Institute, Cary, NC) was used for the combined analysis of variance (ANOVA), with genotypes fixed and environments random. Upon observing significant genotype × environment (G × E) interactions for western corn rootworm damage ratings, we used principal components analysis for damage ratings on environment means using the PROC PRINCOMP procedure of SAS to identify “environment groups” that covaried similarly. ANOVA was then conducted for each environment group to verify that the G × E interaction was no longer significant and that the data for each environment group could be combined.

For each environment group, the phenotypic variance due to genotypes was partitioned into check versus entry, among checks, and among entries following the protocol of Piepho et al. (2006). Similarly, the entry variance was partitioned into inbreds versus hybrids, among inbreds, and among hybrids. Least squares means (LSmeans) were calculated for each environment group and an average least significant difference (LSD) \( P = 0.05 \) was derived by averaging the standard errors of all genotype comparisons created by the pdiff option of the LSMEANS statement of SAS. Pearson’s phenotypic correlations were obtained using the PROC CORR procedure of SAS based on LSmeans for each environment group.

Mid-parent heterosis was calculated for all inbred-hybrid pairs on an environment group mean-basis for each trait according to Hallauer and Miranda Fo (1981):

\[
\frac{(F_1 - MPV)}{MPV}
\]

where \( F_1 \) is the performance of the \( F_1 \) hybrid, and MPV is the mid-parent value (average performance of the two inbred parents). Similarly, best parent heterosis was calculated as follows:

\[
\frac{(F_1 - BPV)}{BPV}
\]

where BPV is the best-parent value (the value of the parent with the lowest damage rating).

Results

Analysis of Variance and Principle Component Analysis. A preliminary ANOVA across all seven environments revealed significant G × E interactions \( (P < 0.05) \) and highly significant tests of genotypes \( (F = 3.36; df = 53, 315; P < 0.001) \) (data not shown). Because of the extensive G × E interactions, we used principle component analysis to determine how the environments covaried for damage ratings. When all seven environments were jointly analyzed, the first (Eigenvalue = 2.79; \( R^2 = 39.9\% \)) and second (Eigenvalue = 1.14; \( R^2 = 16.4\% \)) principle components revealed that the Urbana environment was distinctly different than the other environments (Fig. 1). This is
perhaps not surprising as there are differences in the spatial distribution of rootworm eggs in the soil between the practice of artificial infestation (Brookings and most Missouri environments) and the clumped nature of oviposition in natural infestations (Branson 1986), such as the trap crop situation of Urbana. In addition, Urbana often experiences extreme levels of western corn rootworm infestation that dramatically reduce the variance between genotypes, such that all entries seem to be equally susceptible. Natural egg populations in trap crops in the Urbana area have averaged 9.05 × 10^7 eggs per hectare in past years (Pierce and Gray 2006), which is equivalent to 2.103 eggs per 30.5 cm of maize row, assuming a row spacing of 76.2 cm, nearly double that of our highest artificial infestation level. The second principle component identified a split between environment group 1 (Bradford in 2006 and Poultry Farm in 2007) and environment group 2 (Bradford 1, Bradford 2, Marshall, and Brookings in 2007). Thus, the data set was divided into Urbana, environment group 1, and environment group 2.

Subsequent ANOVA of each environment group showed a lack of significant \((P > 0.05)\) \(G \times E\) interactions (Table 1) and that the data within each environment group could be combined. The test of genotypes was highly significant for environment groups 1 \(F = 3.30; \text{df} = 53, 50; P < 0.001\) and 2 \(F = 2.19; \text{df} = 53, 159; P < 0.001\) but not significant for Urbana \(F = 1.10; \text{df} = 53, 106; P = 0.399\); thus, Urbana was excluded from further analysis.

### Table 1. Mixed model analysis of variance by Environment Group for western corn rootworm damage ratings

<table>
<thead>
<tr>
<th>Random effect</th>
<th>Environment group 1*</th>
<th>Environment group 2*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate</td>
<td>SE</td>
</tr>
<tr>
<td>Env.</td>
<td>0.021</td>
<td>0.033</td>
</tr>
<tr>
<td>Rep(Env.)</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Genotype × Env.</td>
<td>0.021</td>
<td>0.023</td>
</tr>
</tbody>
</table>

* * *, ** *, *** significant at the 0.05, 0.01, and 0.001 probability levels, respectively. Ns, not significant at \(P = 0.05\).


with B73, their hybrids had the two lowest root damage ratings (0.36 ± 0.38 and 0.30 ± 0.38, respectively) for this environment group. Similarly, in environment group 1, Oh7B was one of the least damaged inbred lines (damage rating of 0.61 ± 0.25), but its B73 hybrid was among the most damaged of the hybrids (0.83 ± 0.29) (Table 2).

### Heterosis for Damage Ratings

Mid-parent heterosis, or the performance of the F1 hybrid relative to the mean of the parents, was generally in the negative direction for both environment groups for western corn rootworm damage ratings (Table 3), indicating increased levels of resistance to western corn rootworm larvae feeding in the F1 hybrids. The exceptions were hybrids that were more susceptible than the non-B73 parent. The overall negative heterosis values indicate that hybrids were less damaged than their inbred parents in a nonadditive manner.

For environment group 2, results for best-parent heterosis, or the performance of the F1 hybrid relative to better parent (in this case, the parent with the lower damage rating), follow the same trend as mid-parent heterosis. For environment group 1, however, the results were radically different. In this group of environments, B73 had the lowest damage rating of the inbred lines and served as the best parent in all comparisons. The resulting best-parent heterosis was positive for 15 of the 25 hybrids.

### Discussion

Western corn rootworm feeding damage to diverse inbred lines had no correlation to the feeding damage of B73 hybrids of these same lines (Fig. 2). Therefore, it was not possible to predict the level of resistance in B73 hybrids based on the performance of inbred lines. As mentioned above, highly damaged inbred lines from environment group 2 (CML103 and CML52) were the least damaged as hybrids in the same environmental group (Table 2). Similarly, the inbred line Oh7B had the lowest damage rating for inbreds in environmental group 1, but its B73 hybrid had one of the highest damage ratings.
for hybrids. If we had selected resistant germplasm based solely on inbred performance, we would have missed CML52 and CML103 as potentially important sources of western corn rootworm resistance and would have chosen Oh7B whose resistance would not hold up in hybrid combination. These findings are consistent with prior studies where correlations between inbred and hybrid performance for yield and yield components (e.g., ear length, ear diameter, kernel row number, kernel depth, plant height, days to flower, ear number) were generally low, although variable, across studies (reviewed in Hallauer and Miranda Fo 1981).

Although a healthy, vigorous root system of hybrid maize can probably better withstand attack by insect pests (i.e., tolerance), it is more difficult to envision how hybrid vigor can lead to reduced damage ratings, because root damage ratings are generally associated with antibiosis and/or nonpreference mechanisms. It would be unwise to speculate on how vigor is responsible for reduced feeding because the genetic basis of host-plant resistance to western corn rootworm has not yet been elucidated. The undoubtedly complex relationships among antibiosis, nonpreference, tolerance, and now hybrid vigor have yet to be explored.

At first glance, our results seem to represent an interesting contrast to the findings of Hibbard et al. (1999) in which in a diallel of seven sources of resistance to western corn rootworm larval feeding and two susceptible inbred lines, they found significant general combining ability for six of the entries and very little significant specific combining ability (i.e., heterosis). The six entries that showed significant general combining ability were segregating populations, whereas none of the three inbred lines had significant general combining ability effects. It may be that population crosses show low levels of heterosis for western corn rootworm resistance because the populations themselves show considerable heterosis. It also may be unreasonable to compare these two studies because the current study only evaluated the only F1 hybrids with B73 rather than a complete diallel analysis.

As reviewed by Moeser and Hibbard (2005), several research programs over the past 70 yr have devoted significant effort into identifying and improving maize germplasm for native resistance to western corn rootworm larval feeding. Unfortunately, as of 2008, no commercial hybrid claims native resistance to western corn rootworm larval feeding, though this may be changing soon. Why is it taking so long for sources of native resistance to western corn rootworm larval feeding to reach the market place? The full story is too long and complex to include (see Bohn 2005), but a possible contributing factor is documented here. Inbred susceptibility to larval feeding does not predict hybrid performance. One obvious implication of our data is a need to reconsider methodologies and strategies for screening germplasm for resistance to western corn rootworm. Although recurrent selection with maize populations has resulted in germplasm with reduced western corn rootworm damage (Hibbard et al. 2007), researchers should consider topercrossing inbred germplasm before the evaluation process. It should be stressed that the choice of tester is important; we chose B73, in part because this inbred is the line whose genome is being sequenced, but perhaps
another inbred may be more suitable. Topcrossing all screening materials to form hybrids or population hybrids before western corn rootworm screening would require more time and effort, and could reduce the overall efficiency of the program by extending the screening process by an extra winter season before evaluating the topcrosses. However, taking the extra step of generating hybrid materials would decrease the chance that valuable sources of resistance to western corn rootworm are not missed during the screening process. Resistance identified in any germplasm must hold up in hybrid conditions to be incorporated into commercial products and ultimately be useful to farmers and producers.

Acknowledgments

We thank Julie Barry, Matt Higdon, and Tim Praiswater (USDA-ARS, Columbia, MO), and Dave Schneider and Jeff Olsen (USDA-ARS, Brookings, SD) for assistance in phenotypic evaluations. We also thank Jim Holland (USDA-ARS, Raleigh, NC) and Ana Butrón (Misión Biológica de Galicia, Pontevedra, Spain) for statistical guidance. This research was supported by Hatch funds (University of Illinois) and USDA-ARS.

References Cited


Meinke, L. J., B. D. Siegfried, R. J. Wright, and L. D. Chandler. 1998. Adult susceptibility of Nebraska western corn rootworm (Coleoptera: Chrysomelidae) populations to selected insecticides. J. Econ. Entomol. 91: 594–600.


Pierce, C.M.F., and M. E. Gray. 2006. Western corn rootworm, Diabrotica virgifera virgifera LeConte (Coleoptera: Chrysomelidae), oviposition: a variant’s response to maize phenology. Environ. Entomol. 35: 423–434.


injured by western corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 82: 1760–1763.


Received 3 December 2008; accepted 12 March 2009.