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Short communication

Resistance of Tripsacorn-introgressed maize lines to *Sitophilus zeamais*James E. Throne^{a,*}, Mary W. Eubanks^b^a USDA, Agricultural Research Service, Center for Grain and Animal Health Research, 1515 College Avenue, Manhattan, KS 66502, USA^b Department of Integrative Biology, University of Texas, Austin, TX 78712-1211, USA

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ABSTRACT

The maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), is one of the major pests of maize worldwide. We tested one Tripsacorn-introgressed inbred maize line and 42 hybrid combinations between eleven public inbred lines and 16 different Tripsacorn-introgressed inbreds for resistance to the maize weevil to investigate if there is a genetic basis for resistance to the maize weevil that can be conferred to maize. No progeny were produced in 21 of the entries, and only eight entries had progeny production significantly greater than zero. All the lines that exhibited complete resistance (no progeny produced) are F₁ hybrids between 10 different Tripsacorn-introgressed inbred lines combined with 8 different public maize inbreds. Results indicate that all 16 Tripsacorn-introgressed inbred lines confer resistance in F₁ hybrids. In some of the Tripsacorn-introgressed lines, the degree of resistance expressed varied according to combining ability and heterotic group background. Based on the results, we hypothesize a dominant gene for weevil resistance is inherited from Tripsacorn. The data indicate that Tripsacorn provides a valuable tool for conferring native weevil resistance to maize.

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1. Introduction

Postharvest losses to stored products due to insects can be up to 9% in the U.S. (Phillips and Throne, 2010), and losses of 30–70% or more are common in developing countries (Obeng-Ofori, 2011). The maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), is one of the major pests of maize worldwide. Control of the maize weevil in developing countries is particularly problematic because use of conventional control measures, such as insecticides, may be inadequate (Obeng-Ofori, 2011). Thus, development of alternative control methods that are easier and safer to use with no added expense would be particularly useful in developing countries, as well as in developed countries.

One alternative to the use of insecticides for control of stored-product insects is the use of resistant varieties (Throne et al., 2000). Maize weevil population development and damage to grain can vary greatly with maize variety (e.g., Abebe et al., 2009). Thus, development of maize varieties with resistance to stored-product insects would provide a biorational method for control. Throne and Eubanks (2002) reported that Tripsacorn, a

recombinant developed from crossing a diploid perennial teosinte, *Zea diploperennis* Iltis, Doebley, and Guzmán, and eastern gamagrass, *Tripsacum dactyloides* L., that resembles the earliest known samples of primitive maize, was immune to attack by *S. zeamais*. The hardness of the fruitcase appeared to inhibit oviposition, but this level of hardness probably would not be desirable in a commercial maize variety. Since that study, Tripsacorn-introgressed maize lines have been combined with public inbred lines to select for resistance to western corn rootworm, *Diabrotica virgifera virgifera* LeConte (WCR) (Prischmann et al., 2009). Here, we test hybrids derived from those lines for resistance to the maize weevil to investigate if there is a genetic basis for resistance to the maize weevil that can be conferred to maize.

2. Materials and methods

2.1. Maize lines

We tested two maize lines including a susceptible commercial Asgrow maize line (RX899) used for our maize weevil culture and a resistant proprietary commercial hybrid (HC33 X HC282), one Tripsacorn-introgressed inbred line (SDG-A), and 42 hybrid combinations between 16 different Tripsacorn-*Zea* introgressed inbreds and 11 publicly available inbreds (USDA-AMS Plant Variety Protection Office, 2013) (Tables 1 and 2). The

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Table 1
Inbred lines composing the hybrids tested in the bioassays.

Public inbreds from PVP ^a	Tripsacorn-introgressed inbreds	Composition
FR19	SDG-A	90.63% maize, 9.37% exotic
LH1	SDG-B	93.75 maize, 6.25% exotic
LH38	SDG-C	87.5 maize, 12.5% exotic
LH39	SDG-D	93.75 maize, 6.25% exotic
LH51	SDG-E	87.5 maize, 12.5% exotic
LH74	SDG-F	93.75 maize, 6.25% exotic
LH82	SDG-G	73.44 maize, 26.56% exotic
LH93	SDG-H	73.44 maize, 26.56% exotic
LH119	SDG-I	87.5% maize, 12.5% exotic
LH145	SDG-J	87.5% maize, 12.5% exotic
N196	SDG-K	87.5% maize, 12.5% exotic
	SDG-L	87.5% maize, 12.5% exotic
	SDG-M	87.5% maize, 12.5% exotic
	SDG-N	75% maize, 25% exotic
	SDG-O	95% maize, 5% exotic
	SDG-P	96.87% maize, 3.13% exotic

^a USDA-AMS Plant Variety Protection Office (2013).

introgressed maize lines derive from two *Tripsacum-Zea* recombinant cultivars, Tripsacorn (Eubanks, 1992) and Sun Star (Eubanks, 1996). Both cultivars were initially selected in a bioassay for resistance to western corn rootworm (Eubanks, 2006). Resistant plants from each WCR bioassay were crossed to maize inbred lines B73 (seed provided by Arnel Hallauer at Iowa State University) and W64A (seed provided by James Coors at the University of Wisconsin–Madison). The F₁ generations were selfed, and F₂ (S₁) progeny were tested in another bioassay. Resistant plants from each subsequent bioassay were backcrossed to maize, and the progeny of each cross was selfed. This recurrent cycle of backcrossing, selfing, and selecting for resistance to WCR continued for 14 generations. The introgressed inbred lines in this experiment derive from the series of recurrent selection CRW experiments and maize backcross cycles. The genetic makeup of the Tripsacorn seeds we initially tested for weevil resistance (Throne and Eubanks, 2002) was 50% Eastern gamagrass (female parent) and 50% perennial teosinte (pollen donor). The genetic makeup of the lines in this assay ranged from around 75% maize and 25% exotic (i.e., gamagrass–teosinte recombinant genes) to over 96% maize and approximately 3% exotic (Table 1).

2.2. Bioassays

Maize was cleaned over a U.S. Standard No. 6 sieve (3.35-mm openings). Kernels of each maize line (30.0 ± 0.5 g) were placed in each of five plastic vials (30-mm-diam X 81-mm-high) covered on the top with 100-mesh brass screen. Vials were randomly placed in a plastic box (28 × 38.5 × 15 cm high) with a false floor over a saturated sodium chloride solution to maintain relative humidity at 75% (Greenspan, 1977). An additional container with 300 g of the Asgrow maize was placed in the box to monitor moisture content weekly using a Motomco Model 919 automatic grain moisture tester (DICKEY-john, Auburn, IL). Samples were equilibrated for six weeks and experiments were conducted at 30 °C, which is nearly optimal for maize weevil population development (Throne, 1995).

Maize weevils used were from a culture that originated with weevils collected in or around bins of maize in South Carolina in 1994. Chilled weevils (2–3-week-old) were sexed using snout characteristics (Tolpo and Morrison, 1965), and five females were placed in each vial for 72 h to oviposit. After the weevils were removed from vials, sex and identification were confirmed by examination of genitalia after immersion in ethanol (Whitehead,

1991). Samples were sieved every 3.5 days, starting 15 days after ovipositing females were removed and continuing until no weevils had emerged in each vial for 2 weeks.

Number of progeny produced and development time were analyzed using analysis of variance (PROC GLM, SAS Institute, 2008). For the analysis of number of progeny produced, we excluded data from treatments that had no progeny produced because variances for these data cannot be homogenized with variances for treatments that had non-zero values. We then used a one-tailed *t*-test to determine whether number of progeny produced on treatments with non-zero values differed from zero progeny produced on the treatments not included in the analysis of variance. For the analysis of development time, we only used data for lines that had progeny produced in at least three replications.

3. Results and discussion

No progeny were produced in 21 of the entries, and mean number of progeny produced was one or fewer in 16 of the entries (Table 2). More progeny were produced on our culture maize, entry 06-45, than on any other entry. Entries 06-4, -5, -7, -9, -10, -43, -44, and -45 were the only entries with progeny production significantly greater than 0. For entries that had at least three replications with progeny produced (entries 06-5, -6, -7, -10, and -45), there were no differences in development time (data not shown, but mean development time for these entries ranged from 38.9 to 42.5).

All the lines that exhibited complete resistance (no progeny produced) are F₁ hybrids between 10 different Tripsacorn-introgressed (SDG) inbred lines combined with 8 different public inbred lines. Unlike these lines, progeny were produced on the resistant control. Nine SDG lines (06-10, 06-7, 06-5, 06-6, 06-4, 06-9, 06-43, 06-8, and 06-44) had a numerically greater number of weevils produced than in the resistant control, although progeny production on only two of these lines was significantly greater than on the resistant control (06-10 and 06-7). With the exception of 06-4, which is an inbred line, these nine SDG lines are F₁ hybrids of Tripsacorn-introgressed inbred lines crossed with public inbreds. These data indicate that all 16 Tripsacorn-introgressed inbred lines confer resistance in F₁ hybrids. In some Tripsacorn-introgressed lines, the degree of resistance expressed varied according to combining ability and heterotic group background. For example, there were 6.0 weevils produced

Table 2

Hybrid combinations for the lines tested, mean (\pm SE) number of weevils produced on each line, and *t*-test to determine if number of weevils produced was significantly greater than zero.

Entry no.	Line	Number of weevils ^a	<i>t</i>	<i>P</i>
06-45	Susceptible control	14.6 \pm 2.8a*	5.2	0.0033
06-2	Resistant control	0.6 \pm 0.4d	1.5	0.1040
06-10	SDG-A X LH93 (F ₂ /S ₁)	10.0 \pm 2.8 ab*	3.5	0.0123
06-7	LH93 X SDG-I	7.8 \pm 2.6bc*	3.0	0.0199
06-5	LH39 X SDG-H	6.4 \pm 2.1bcd*	3.1	0.0181
06-6	LH38 X SDG-B	6.0 \pm 3.2bcd	1.9	0.0653
06-4	SDG-A	2.4 \pm 1.1cd*	2.1	0.0497
06-9	LH145 X SDG-A	2.4 \pm 1.0cd*	2.3	0.0401
06-43	LH119 X SDG-O	1.0 \pm 0.4d*	2.2	0.0445
06-8	FR19 X SDG-D	0.8 \pm 0.8d	1.0	0.1870
06-44	LH119 X SDG-N	0.8 \pm 0.4d*	2.1	0.0497
06-23	LH51 X SDG-C	0.6 \pm 0.6d	1.0	0.1870
06-38	LH119 X SDG-K	0.6 \pm 0.4d	1.5	0.1040
06-41	LH38 X SDG-G	0.4 \pm 0.2d	1.6	0.0889
06-3	SDG-I X SDG-B	0.2 \pm 0.2d	1.0	0.1870
06-25	LH39 X SDG-C	0.2 \pm 0.2d	1.0	0.1870
06-40	SDG-E X LH145	0.2 \pm 0.2d	1.0	0.1870
06-35	LH1 X SDG-G	0.2 \pm 0.2d	1.0	0.1870
06-36	SDG-H X LH145	0.2 \pm 0.2d	1.0	0.1870
06-34	LH119 X SDG-L	0.2 \pm 0.2d	1.0	0.1870
06-27	LH38 X SDG-M	0.2 \pm 0.2d	1.0	0.1870
06-37	LH119 X SDG-M	0.2 \pm 0.2d	1.0	0.1870
06-28	LH119 X SDG-K	0.2 \pm 0.2d	1.0	0.1870
06-32	SDG-D X SDG-A	0.2 \pm 0.2d	1.0	0.1870
06-16	SDG-A X LH93 (F ₁)	0 \pm 0.0		
06-33	LH93 X SDG-A	0 \pm 0.0		
06-20	SDG-A X LH145	0 \pm 0.0		
06-29	LH145 X SDG-B	0 \pm 0.0		
06-39	LH93 X SDG-B	0 \pm 0.0		
06-24	LH145 X SDG-C	0 \pm 0.0		
06-31	LH38 X SDG-C	0 \pm 0.0		
06-12	SDG-D X LH82	0 \pm 0.0		
06-14	LH39 X SDG-D	0 \pm 0.0		
06-18	SDG-D X SDG-F	0 \pm 0.0		
06-19	SDG-D X LH145	0 \pm 0.0		
06-22	LH145 X SDG-D	0 \pm 0.0		
06-42	SDG-D X LH119	0 \pm 0.0		
06-26	LH74 X SDG-P	0 \pm 0.0		
06-21	LH145 X SDG-F	0 \pm 0.0		
06-11	LH119 X SDG-F	0 \pm 0.0		
06-15	LH93 X SDG-F	0 \pm 0.0		
06-1	N196 X SDG-H	0 \pm 0.0		
06-17	LH74 X SDG-I	0 \pm 0.0		
06-30	LH39 X SDG-J	0 \pm 0.0		
06-13	LH74 X SDG-M	0 \pm 0.0		

Means followed by an * are significantly greater than 0 (one-tailed *t*-test, *df* = 4, *P* < 0.05).

^a Means followed by the same letter are not significantly different (*F* = 8.4, *df* = 23, 96; *P* < 0.0001; Ryan–Einot–Gabriel–Welsch multiple range test, *P* > 0.05).

on entry 06-6 (LH38 X SDGB), but no weevils were produced on entries 06-29 (LH145 X SDGB) or 06-39 (LH93 X SDGB). LH145 and LH93 are both from the stiff stalk synthetic maize heterotic group;

whereas, LH38 is non-stiff stalk. We hypothesize a dominant gene for weevil resistance is inherited from *Tripsacorn* because entry 06-10 is the segregating F₂ generation of entry 06-16 which had no weevils produced. Our hypothesis is further supported by the fact that 06-33, which is the F₁ reciprocal cross of 06-16, also had no weevil emergence. These experimental data indicate that *Tripsacorn* provides a valuable tool for conferring native weevil resistance to maize.

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