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## Resistance of Tripsacorn to *Sitophilus zeamais* and *Oryzaephilus surinamensis*

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### Abstract

One strategy that has been used to find germplasm for developing improved plant varieties is to test ancestral germplasm for the desired traits. Although the progenitors of commercial maize are not known, a hybrid (called Tripsacorn) developed from a perennial teosinte, *Zea diploperennis*, and eastern gamagrass, *Tripsacum dactyloides*, resembles the earliest known samples of primitive domesticated maize. We tested resistance of whole Tripsacorn to the primary storage pest (primary storage pests can infest intact kernels) the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), and resistance of ground Tripsacorn to the secondary storage pest (secondary pests usually cannot infest intact kernels) the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae). Tripsacorn was immune to attack by *S. zeamais*. The weevils were unable to lay eggs in the Tripsacorn, and we hypothesized that the hardness of the fruitcase was responsible for lack of weevil oviposition. *Oryzaephilus surinamensis* were able to complete immature development on ground Tripsacorn, but duration of development was longer and weight of emerged adults was less than for beetles developing on wheat. Hardness of the fruitcase may have been a primitive mechanism of defense against insects and other pests, but probably would not be an acceptable trait in commercial varieties. It remains to be determined whether the possible antibiotic effect demonstrated in ground Tripsacorn would be a useful trait in commercial maize hybrids. © 2002 Elsevier Science Ltd. All rights reserved.

**Keywords:** Host plant resistance; *Oryzaephilus surinamensis*; *Sitophilus zeamais*; *Tripsacum dactyloides*; Tripsacorn; *Zea diploperennis*

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## 1. Introduction

Losses of stored grains due to insects and other storage problems are estimated to be greater than US\$ 1 billion annually in the United States (Cuperus and Krischik, 1995) and may exceed 30% in developing countries. Residual insecticides have been used routinely for many years to control insect pests in stored grain. However, use of residual insecticides is becoming less desirable because of development of resistance by insects to insecticides, regulatory restrictions on use of insecticides, and consumer desire for a pesticide-free product (Arthur, 1996). One alternative control technology that has been largely ignored since the widespread use of residual insecticides on stored grain is resistance of grains to insects. Most new grain varieties are selected for agronomic traits, such as yield, but not for resistance to pests in storage (Throne et al., 2000).

One strategy that has been used to find germplasm for developing improved plant varieties is to test ancestral germplasm (germplasm from progenitors of commercial varieties) for the desired traits. For example, Arnason et al. (1994) showed that some Mexican landraces of maize, *Zea mays* L., were resistant to maize weevils, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), and that the resistance was correlated with phenolic acid content of the maize. There are many theories about the possible origin of maize (MacNeish and Eubanks, 2000), with hypothesized progenitors including an extinct wild maize and maize's closest extant wild relatives, teosinte and *Tripsacum*. A previously unknown wild relative of maize, *Zea diploperennis* Iltis, Doebley, and Guzmán, was discovered in the late 1970's in the mountains of Jalisco, Mexico (Iltis et al., 1979). *Zea diploperennis* is a perennial variety of teosinte. Eubanks (1992, 1995) was able to cross *Zea diploperennis* with eastern gamagrass, *Tripsacum dactyloides* (L.), supporting the hypothesis that *Tripsacum* could have played a role in the origin of maize. The hybrid closely resembles the earliest known samples of primitive domesticated maize (Eubanks, 1995). The cross with *Tripsacum* as the female parent is referred to as Tripsacorn (Eubanks, 1992). The kernels of Tripsacorn, like those of its parents, are enclosed in a hard, armor-like fruitcase [for a morphological description of the cupulate fruitcase, see Galinat (1970)]. The hybrid can be used as a genetic bridge for transferring *Tripsacum* genes into maize.

Given that Tripsacorn may be similar to the progenitors of modern maize and that Tripsacorn is resistant to corn rootworms (Eubanks, 1993), we initiated studies to determine whether Tripsacorn may have resistance to storage insect pests that could be incorporated into commercial maize hybrids. We tested resistance of intact Tripsacorn kernels to the maize weevil, a primary pest that can damage intact grain kernels. Based on results of that study, we also tested whether the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae), a secondary pest that cannot damage intact grain kernels, could oviposit and complete development from egg to adult on ground Tripsacorn.

## 2. Materials and methods

### 2.1. Resistance of whole kernels to maize weevils

Tripsacorn was obtained from Sun Dance Genetics (Durham, NC). Asgrow RX899 maize used for our maize weevil culture was included as a control to ensure that the weevils used in the

Tripsacorn study were capable of laying viable eggs that could develop to adults. Tripsacorn was cleaned over a US Standard No. 8 sieve (2.38 mm openings), and the maize was cleaned over a US Standard No. 6 sieve (3.35 mm openings). We used different sizes of sieves because the Asgrow RX899 maize kernels are larger than the Tripsacorn kernels. We X-rayed (Throne, 1995) all kernels to ensure that they were not previously infested with insects. For X-raying, kernels were placed in a single layer on sheets of cellulose (previously exposed 13 × 18 cm radiographs) that were coated with double-stick tape. A sheet of kernels was placed on a sheet of film (Kodak Industrex M X-ray film in Ready Pack II foil packs, Eastman Kodak, Rochester, NY) placed 56 cm below an X-ray source (Model 43855A Faxitron, Hewlett–Packard, McMinnville, OR) and exposed at 18 kV and 3 mA for either 3 minutes for Tripsacorn or 4 minutes for maize. Negatives were examined under a stereomicroscope at a minimum of 12 × magnification for the presence of any insect stage.

Kernels of maize or Tripsacorn ( $35.0 \pm 0.1$  g) were placed in each of three plastic cages (30 mm diam × 81 mm high) covered on the top with 100-mesh brass screen. Cages 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 within the box at 75% (Greenspan, 1977). An additional cage containing 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). The samples were equilibrated for six weeks at 30°C. These environmental conditions are nearly optimal for maize weevil population development (Throne, 1995) and were maintained throughout the study.

Maize weevils for the study were from a culture that originated with weevils collected in or around bins of maize in South Carolina in 1994 and were F<sub>10–11</sub> laboratory generation. The cultures were maintained on maize (14–15% moisture content) at 25°C. Chilled weevils (2–3-week-old) were sexed using snout characteristics (Tolpo and Morrison, 1965), and five females were placed in each cage for 72 h to oviposit. After the weevils were removed from cages, sex and identification of these females were confirmed by examination of genitalia after immersion of weevils in ethanol (Whitehead, 1991). Samples were sieved daily, starting 13 days after ovipositing females were removed and continuing until no weevils had emerged in each cage for 2 weeks. Samples were again X-rayed after emergence was complete to determine whether there were dead insects in kernels.

## 2.2. Resistance of ground kernels to sawtoothed grain beetles

Because weevils did not oviposit in Tripsacorn in the first study, we conducted a second study to determine whether ground Tripsacorn could support insect development. We used *O. surinamensis* in the study, rather than maize weevils, because *O. surinamensis* readily complete development in ground grain whereas maize weevils do not (Meagher et al., 1982). We used hard red winter wheat (*Triticum aestivum* L.) of unknown variety as a control to ensure that the beetles used in the study were capable of laying viable eggs that could develop to adults. We used wheat as a control, rather than maize, because we wanted to provide each insect with an entire kernel to ensure that all parts of the kernel were available for oviposition and feeding. Tripsacorn kernels are small and closer in weight to wheat than maize. We would have needed many more Tripsacorn kernels if we had used one maize kernel and a comparable weight of Tripsacorn kernels for each

replication, and we had a limited supply of Tripsacorn kernels. Development of a related species, the merchant grain beetle [*O. mercator* (Fauvel)], is similar on maize and wheat (LeCato, 1974). We selected 20 Tripsacorn kernels and 20 pairs of wheat kernels that weighed between 58 and 70 mg (i.e., a Tripsacorn kernel weighs twice as much as a wheat kernel, so we used two wheat kernels to approximate the weight of one Tripsacorn kernel). Kernels were individually ground (pairs for wheat) with a mortar and pestle, and the resulting material was placed in a 0.5 ml microcentrifuge tube with a pinhole in the lid. Tubes, with the lids open, were randomly placed in a plastic box over sodium chloride at 30°C to equilibrate for 3 weeks. These environmental conditions are nearly optimal for population development of sawtoothed grain beetles (Howe, 1956) and were maintained throughout the study.

Several hundred unsexed sawtoothed grain beetles, from a colony that has been maintained at the Grain Marketing and Production Research Center for many years on rolled oats containing small amounts of wheat germ and brewers' yeast and reared at 25°C and 60–65% r.h., were placed in a pint (ca. 473 ml) jar containing wheat flour and allowed to oviposit for 24 hours. Eggs were sieved from the flour over US Standard No. 70 and 30 sieves (212 and 600 µm openings, respectively). A single egg was placed in each microcentrifuge tube using a fine brush. Starting 15 days after eggs were placed in tubes, tubes were examined every 3.5 days (without disturbing the contents) for an adult.

We noticed that progeny reared on Tripsacorn appeared to be smaller than those reared on wheat, so we conducted an additional test in which emerged adults were chilled in a refrigerator (ca. 3°C) shortly after emergence and then weighed. Methods were modified slightly from those used in the previous study by increasing the number of Tripsacorn kernels or pairs of wheat kernels to 30; collecting eggs for 24 h at 30°C; and starting to check tubes daily for emerged adults 17 days after eggs were placed in tubes.

### 3. Results

#### 3.1. Resistance of whole kernels to maize weevils

No weevils were produced on the Tripsacorn, and X-ray examination of the kernels indicated that no eggs had been laid in the kernels. Mean ( $\pm$ SEM) progeny production on the Asgrow maize was  $30.3 \pm 3.7$  ( $n = 3$ ), which was significantly different from 0 ( $t = 8.2$ ,  $P = 0.01$ ), demonstrating that weevils used in the study were capable of laying viable eggs that could develop to adults. Moisture content of the 300-g sample of Asgrow maize ranged from 14.1 to 14.7%, wet weight, during the study ( $\bar{x} \pm \text{SEM} = 14.4 \pm 0.05\%$ ).

#### 3.2. Resistance of ground kernels to sawtoothed grain beetles

*O. surinamensis* were able to oviposit and complete development from egg to adult on ground Tripsacorn. Survivorship on Tripsacorn was similar to that on wheat—11 and 12 (of 20) adults emerged on wheat and Tripsacorn, respectively, in the first test, and 14 and 16 (of 30) adults emerged on wheat and Tripsacorn, respectively, in the second test. Duration of development on

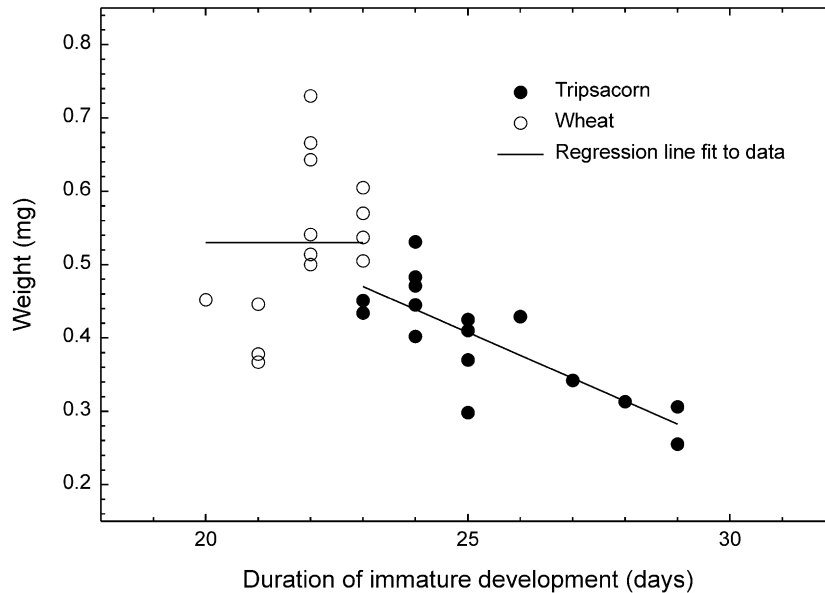


Fig. 1. Relationship between adult weight and duration of immature development of sawtoothed grain beetles that developed from eggs placed in ground wheat or Tripsacorn. Wheat: weight (mg) =  $0.53 (\pm 0.03)$ . Tripsacorn: weight (mg) =  $1.19 (\pm 0.16) - [0.0313 (\pm 0.0063) \times \text{days}]$ . Numbers in parentheses are standard errors of the equation parameters.

Tripsacorn was similar to that on wheat in the first test ( $\bar{x} \pm \text{SEM} = 24.5 \pm 0.0$  days vs.  $24.5 \pm 0.5$  days, respectively). However, when adults were removed daily in the second test (rather than every 3.5 days in the first test), duration of development was longer on Tripsacorn than on wheat ( $25.3 \pm 0.5$  vs.  $21.9 \pm 0.2$  days, respectively;  $F = 35.0$ ;  $df = 1, 29$ ;  $P < 0.01$ ). Weight of emerged adults on Tripsacorn was less than on wheat ( $0.40 \pm 0.02$  mg vs.  $0.53 \pm 0.03$  mg, respectively;  $F = 16.4$ ;  $df = 1, 29$ ;  $P < 0.01$ ). Beetles emerging first on Tripsacorn weighed more than those that emerged later (Fig. 1;  $r = -0.80$ ,  $P[r > 0] < 0.01$ ). Weight and emergence time were not correlated on wheat ( $r = 0.52$ ,  $P[r > 0] = 0.055$ ).

#### 4. Discussion

Whole Tripsacorn kernels were immune to attack by maize weevils. The Tripsacorn kernels were difficult to grind because of the hardness of the fruitcase, and weevils probably were unable to lay eggs in the Tripsacorn because of the hardness of the fruitcase. It is also possible that there is a repellent in the fruitcase that deters oviposition. Protection of the fruit by the fruitcase may have been a primitive method of defense against insects and other pests. When protection of the fruit by the fruitcase was compromised by grinding, *O. surinamensis* were able to oviposit and complete development from egg to adult on Tripsacorn. This does not support the hypothesis that there may be a repellent in the fruitcase, unless the repellent is inactivated by grinding. It seems more likely that the hardness of the fruitcase protects the fruit. Thus, Tripsacorn is not immune to

insect attack once the fruitcase is compromised. Primary pests that are strong borers, such as the larger grain borer [*Prostephanus truncatus* (Horn)] or the lesser grain borer [*Rhyzopertha dominica* (F.)], may be able to damage intact Tripsacorn kernels. However, secondary pests would be unlikely to damage intact Tripsacorn kernels.

Another feature of grain morphology that provides a protective barrier is the pericarp that surrounds the fruit. Pericarp hardness has been associated with resistance to maize weevils (e.g., Classen et al., 1990). Pericarp morphology probably does not contribute to resistance in Tripsacorn because the thickness and number of cell layers in the pericarp is significantly less in the wild relatives than it is in maize (Tracy and Galinat, 1987). In *Tripsacum* and teosinte, pericarp thickness ranges from 25 to 30  $\mu\text{m}$  and there are only two to three cell layers; whereas in maize, pericarp thickness ranges from 57 to 182  $\mu\text{m}$  and numbers of cell layers range from 5 to 21. There is little evolutionary impetus for the development of a hard pericarp in *Tripsacum* and teosinte to protect from insect attack if their fruitcases provide immunity to insect attack, as in Tripsacorn.

Weight of *O. surinamensis* progeny developing on ground Tripsacorn was lower than for those developing on ground wheat. [Although we did not test development of *O. surinamensis* on ground maize, weights of *O. mercator* progeny developing on maize and wheat are similar (LeCato, 1974).] This suggests that Tripsacorn kernels may impair insect development, and thus may have an inherited property that could provide protection from insects capable of penetrating the hard fruitcase. Evidence of antibiosis (higher mortality and reduced larval size) in Tripsacorn has been shown with corn rootworms (Eubanks, 1994, 1998). While we do not know the mechanism of antibiotic resistance in Tripsacorn, one possible chemical defense response in grain is production of proteinase inhibitors with insecticidal activity (Reeck et al., 1997). Although there was no apparent correlation between proteinase inhibition and rootworm resistance in Tripsacorn (Eubanks and Cook, 2000), there was a wide range of inhibitory activity in individual maize  $\times$  Tripsacorn plants for every line tested. Therefore, if there is antibiotic resistance in Tripsacorn, it should be possible to select plants with high levels of antibiotic activity and transfer the trait from Tripsacorn to maize in a recurrent selection breeding program.

The commercial potential of Tripsacorn or of hybrids produced from Tripsacorn for food and/or feed use has not been determined. However, the hardness of the fruitcase will have to be bred out in the development of commercial varieties and this probably will eliminate the immunity demonstrated to maize weevils. Thus, this probably is not a useful trait for development of new maize hybrids with resistance to storage insect pests. But, if Tripsacorn can impart an antibiotic effect, this may be important for the production of maize seed with resistance to insects that cause significant grain losses during storage. Further selection may result in Tripsacorn hybrids with antibiotic resistance to storage insect pests.

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