

Research Application Summaries

Corn–Sorghum

Grain Yield of Initial Bt Corn Hybrid Introductions to Farmers in the Northern Corn Belt

Joe Lauer and John Wedberg

Research Question

Losses due to European corn borer (ECB) exceed \$1 billion in the USA annually. Feeding disrupts physiological processes in the plant, leading to lower plant yield as well as plant lodging and ear drop. In 1993, Ciba-Geigy reported transformation of corn inbreds with genes derived from *Bacillus thuringiensis* spp. *kurstaki* (Bt). Since then seed companies have incorporated Bt genes into elite corn hybrids. Little information is available on the grain yield of Bt hybrids in relation to current commercially grown hybrids. Our objectives were to compare Bt hybrid yield with adapted high yielding non-Bt hybrids, and to evaluate Bt control of ECB under economically significant infestations.

Literature Summary

Control of ECB has traditionally been achieved through genetic, chemical insecticides and biological methods. Plant breeders have concentrated on feeding resistance to plant tissue, DIMBOA concentration, and general standability. Effective use of insecticides to control ECB requires scouting and knowledge of treatment thresholds, and immediate application in a relatively short period of time during crop development. Biological methods for controlling ECB include use of various Bt sprays and granules and the release of natural insect enemies. A new tool for controlling ECB is the successful development of corn transformed with genes derived from Bt.

Study Description

Experiments were established at three locations in 1995 and one in 1996. Three groups of corn hybrids were evaluated: Bt hybrids that had been transformed to contain the Bt gene, closely related "isoline" hybrids that did not have the Bt gene, and "standard" hybrids that have historically yielded well at these locations. ECB infestation treatments consisted of inoculation four times during the growing season with 100 ECB eggs or larvae per plant, natural ECB infestation, and insecticide application protecting plots from infestation by feral moths.

Applied Questions

How does the yield of initial Bt hybrid introductions compare with adapted commercial hybrids?

Yield of Bt hybrids was unaffected by ECB infestation. Under natural infestation, Bt hybrids yielded similarly to standard hybrids (Fig. 1). Inoculation of standard hybrids with ECB eggs or larvae decreased yield 4% to 154 bu/acre compared with Bt hybrids. Application of an insecticide to the standard hybrids increased yield to 173 bu/acre, an 8% increase over that of Bt hybrids grown under natural infestation.

Full scientific article from which this summary was written begins on page 373 of this issue.

When and where should Bt corn be used?

The current cost per acre for Bt hybrid seed is equivalent to the cost of applying an insecticide, however, growers have to consider the long-term potential for economic infestations of ECB. The benefit of Bt hybrids is realized during seasons or in areas of consistently high ECB damage. There are good reasons not to grow Bt hybrids in areas where they are not of potential benefit. First the cost of the seed premium will not economically justify the use of Bt hybrids. Since standard non-Bt hybrids yielded as well as or better than Bt hybrids in the absence of infestation, Bt seed may not be worth the additional cost where there is a low probability of infestation. Second, the widespread use of Bt hybrids raises concerns about ECB developing resistance to Bt.

How should Bt corn hybrids be 'selected' by farmers?

Bt hybrids do not necessarily have the most yield potential in every location. Our results indicate that yields of initial Bt hybrid introductions were lower than contemporary elite adapted hybrids in the absence of ECB. Breeding programs will most probably improve the yield of Bt hybrids as new Bt genes are incorporated into the breeding cycles of elite inbreds earlier in the breeding cycle. Hybrid selection by farmers should still be based upon consistent yield performance over an area of wide adaptation and many management practices.

Recommendation

Bt hybrids show significant potential to protect yield under heavy ECB damage and are recommended for use in situations where such infestations may occur. Economic considerations and concerns about the development of resistance dictate that the hybrids be used only in situations where high yield loss is expected, and used in accordance with strategies designed to deter resistance development. We do not recommend use of Bt hybrids in situations where ECB damage is not expected. Unnecessary use of these hybrids will foster development of resistance shortening the useful life of this product, an important production tool. Seed companies, educators and farmers must carefully communicate and deploy strategies to minimize resistance development so that the Bt technology is successful over a long time.

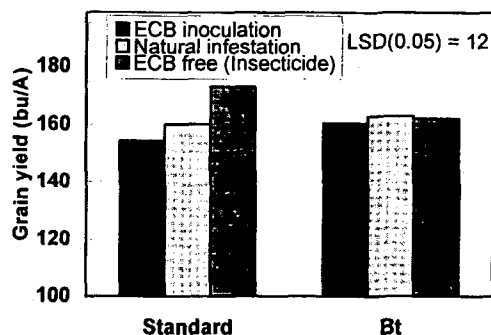


Fig. 1. Grain yield of standard and Bt corn hybrids infested with ECB during 1996 at Arlington, WI.

Research

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Grain Yield of Initial Bt Corn Hybrid Introductions to Farmers in the Northern Corn Belt

Joe Lauer* and John Wedberg

European corn borer (ECB; *Ostrinia nubilalis* Hubner), is a major pest of corn (*Zea mays* L.) in North America. Recently, seed companies have begun to offer control of this pest by introducing synthetic genes derived from *Bacillus thuringiensis* spp. *kurstaki* (Bt) into the corn genome. Our objectives were to compare the yield of Bt hybrids with adapted high yielding non-Bt hybrids, and to evaluate Bt hybrid yield under economically significant ECB infestation. Experiments were established in the field at three locations in 1995 and one location during 1996. Three groups of corn hybrids were evaluated: transformed hybrids with the Bt gene, closely related "isoline" hybrids without the Bt gene, and "standard" high yielding hybrids adapted to these locations. ECB infestation treatments consisted of natural infestation, inoculation four times during the growing season, and insecticide application resulting in an ECB "free" treatment. Grain yield of Bt corn hybrids was not affected by ECB. Yield of isoline hybrids was 10% lower than both standard and Bt hybrids regardless of ECB treatment. Yield of Bt hybrids was 4 to 8% greater than standard hybrids when inoculated with ECB. However, yield of Bt hybrids was 8% less than standard hybrids when an insecticide was applied. Yield of initial Bt hybrid introductions was equivalent to or better than standard hybrids, except in environments with low ECB.

EUROPEAN CORN BORER was introduced into the USA from Europe about 1915 in shipments of infested corn to Massachusetts, New York, and Ontario, and has since spread to become a major pest of corn in North America (Caffery and Worthley, 1927). Yield losses of 2.8 to 7.5 % for each borer on a plant can result from feeding at various stages of plant growth (Calvin et al., 1988). Corn yield reduction from ECB is estimated to exceed \$50 million in Illinois and \$1 billion in the USA annually (Mason et al., 1996). Feeding results in physiological disruption of the plant leading to lower yield as well as plant lodging and ear drop.

ECB can survive the winter as full-grown larvae in weeds or corn stalks. In the Northern Corn Belt, they pupate in

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May, and the moths begin flying in early June and lay eggs on the underside of corn leaves (Wedberg, 1996). The eggs begin to hatch by late June and give rise to first-generation larvae. It takes approximately 22 d to reach the full-grown (fifth instar) stage, and in areas with bivoltine populations the larvae will pupate. The resulting moths will lay a second-generation of eggs that hatch by early August.

Control of ECB has traditionally been achieved through genetic, chemical and biological methods. Plant breeding methods have concentrated on feeding resistance to plant tissue (Hallauer et al., 1988), DIMBOA concentration (Dicke and Guthrie, 1988), and general standability (Duvick, 1984). Effective use of insecticides to control ECB requires scouting and knowledge of treatment thresholds. Once the threshold is reached, applications should be immediate since a relatively narrow application window exists. Many insecticides are registered for ECB control.

Biological methods for controlling ECB include release of natural insect enemies (Dicke and Guthrie, 1988) and spray products containing proteins derived from the ubiquitous soil bacteria, *Bacillus thuringiensis* spp. *kurstaki* (Bt), that forms a crystal protein that is toxic to many insect species (Hofte and Whiteley, 1989). After the insect eats Bt, the crystal dissolves to release a toxin that attacks the gut lining. Feeding stops within a few hours (Sutter and Raun, 1967). The insect gut wall breaks down within 24 h. Bacterial spores germinate and invade the body cavity of the insect. The insect dies from toxins attacking the gut wall, by a general body infection (septicemia) that is present within 48 h, and food deprivation. Over 70 different toxins are formed from Bt crystal proteins. The activity of the toxin in an insect depends on gut pH, the presence of enzymes and reducing agents, and the presence of binding sites on cell membranes.

The first use of Bt products against ECB occurred in Europe in 1929 (Husz, 1929). Currently, many commercial formulations of Bt products are registered. Bt products are effective against first-generation ECB because the larvae concentrate in the leaf whorl of the corn plant, and the product is easy to apply. Bt products are safe not only for applicators and detasslers, but also birds, amphibians, fish, and aquatic invertebrates. Most beneficial insects are not affected by applications aimed at ECB. Use of Bt products

Abbreviations: ECB, European corn borer.

requires early detection and scouting and prompt treatment is critical. Bt activity is relatively short, with UV light and rainfall posing the biggest challenge to persistence (Dunkle and Shasha, 1988). Bt products perform poorly against second-generation larvae and are generally not labeled for use.

Bt corn is a new tool for controlling ECB. In 1993, Ciba-Geigy reported successful Bt transformation of elite corn hybrids (Koziel et al., 1993). Many seed companies have incorporated Bt genes into elite corn hybrids. Incorporation of the Bt toxin into a corn plant overcomes many difficulties associated with insecticides and Bt products.

Depending upon the transformation "event," Bt corn offers 99% control against first generation ECB, but control differs among Bt events against late-season ECB infestation (Ostlie et al., 1997). Bt corn offers potential for cross protection against other caterpillar pests attacking corn. Insecticide applications are eliminated, which may reduce scouting costs and toxicity potential to non-target species due to drift, poor timing, and availability of applicator or product. Little information is available on the yield of Bt hybrids in relation to current commercially grown hybrids. Our objectives were to compare the yield of Bt hybrids with adapted high yielding non-Bt hybrids, and to evaluate Bt hybrid yield under economically significant ECB infestation.

MATERIALS AND METHODS

During 1995, experiments were established near Arlington, Hancock, and Lancaster, WI. The experimental design was a randomized complete block in a split-plot arrangement with four replications. Main plots were hybrid. Three groups of hybrids were evaluated. The Bt events that were registered at the time of this study were 176 and BT11 (Ostlie et al., 1997). Hybrids with the Bt gene included 'Northrup King 1032BT', 'Northrup King 1033BT', 'Ciba 1134E', 'Ciba 3206E' and 'Ciba 3208E'. Closely related isolines that did not have the Bt gene included 'Northrup King 397', 'Northrup King 777', 'Ciba 1134X', 'Ciba 3206X' and 'Ciba 3208X'. Standard hybrids that have yielded well at these locations included 'DeKalb DK512' and 'Golden Harvest H2387'. Split-plots were two ECB treatments: natural infestation and inoculation. Plots were planted on 15 May, 11 May, and 17 May and harvested on 18 Oct., 16 Oct., and 14 Oct. for Arlington, Hancock, and Lancaster, respectively.

During 1996, the experiment was established at Arlington, WI. The experimental design was a randomized complete block in a split-plot arrangement with four replications. Main plots were hybrid. Bt hybrids included: 'Ciba 1401E', 'Ciba 1402E', 'Ciba 4410E', 'Ciba MAX101', 'Ciba MAX21', 'Ciba MAX23', 'Ciba MAX357', 'Ciba MAX454', 'Ciba MAX747', 'Ciba MAX88', and 'Northrup King 4734CBR', 'Northrup King X4334CBR'. Standard hybrids included 'Dairyland 1407', 'DeKalb DK493', 'Golden Harvest H2411', and 'Pioneer 3769'. Split-plots were ECB infestation levels of inoculation, natural infestation, and "ECB free" using the insecticide permethrin, [(3-Phenoxyphenyl) methyl (\pm) cis-trans 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate], Pounce 3.2EC (FMC Corporation, Philadelphia, PA). This latter

treatment was applied to protect plots from infestation by progeny of feral moths and simulate years when ECB injury is low or nondetectable. Moth flights were monitored using a blacklight trap to time the insecticide applications that occurred on 3 July, 21 Aug., and 3 Sep. 1996. Plots were planted on 25 Apr. and harvested on 11 Nov.

In both years, split-plots were four 30-in. rows 25 ft long established at a density of 30 000 plants/acre. Typical cultural practices used by farmers in the surrounding area were used for plot establishment and management. The center two rows were harvested with a plot combine.

ECB inoculations were made on each plant in the two harvested rows to simulate first- and second-generation infestations. First-generation infestation was simulated by using a forceps to insert approximately 100 eggs/plant (laid on waxed paper) deeply into the leaf whorls at V7 and V9. For second-generation infestation, eggs laid on wax paper were pinned to the midrib of the lower surface of the ear leaf at VT and R1 (Guthrie, 1987). In 1996, second-generation inoculation (approximately 100 larvae/plant) at VT and R1 was achieved by using a "bazooka" (Wiseman et al., 1980), which delivered neonate larvae mixed with corn grits directed towards the leaf axil of the ear leaf.

On 11 July 1995 and 5 Aug. 1996 all leaves on 10 consecutive plants in each plot row were examined for damage. A rating scale of 1 to 9 (Guthrie et al., 1960) was used to express the degree of leaf feeding injury that resulted from pretassel infestations. On 17 Oct. 1995 and 19 Oct. 1996, five consecutive plants in a plot were split longitudinally and examined for cavities resulting from stalk tunneling activities of the borer. Cavities were measured and recorded so that a 1-in. length of tunneling equaled one cavity.

Data were analyzed using SAS (1985) procedures for analysis of variance. Treatment mean comparisons were made using least significant difference when *F* were significant ($P \leq 0.05$). The chi-square test (Gomez and Gomez, 1984) was used to verify homogeneity of variance and data were combined.

RESULTS AND DISCUSSION

In 1995, a severe second-generation ECB infestation occurred statewide. In 1996, a severe first-generation ECB infestation was observed statewide.

In 1995, no location \times hybrid interaction was observed. No apparent ECB damage differences were seen between Bt events. Inoculation of ECB decreased grain yields 11 bu/acre at Arlington, and 20 bu/acre at Hancock. Lancaster grain yield was not significantly affected by inoculation.

Less stalk damage and lodging were observed with the Bt hybrids than with the standard and isolate hybrids (Table 1). Averaged across all locations, yield of non-Bt isolate hybrids averaged 147 bu/acre under natural infestation and decreased with inoculation 9% to 134 bu/acre (Fig. 1). Inoculation of standard hybrids with ECB four times during the season decreased grain yields 8% to 146 bu/acre. Standard hybrids yielded 158 bu/acre under natural infestation. Bt corn hybrids, whether inoculated with ECB or not, also yielded 158 bu/acre. Bt corn hybrids yielded similarly to the standard hybrids under the ECB infestation of 1995. Because ECB infestation during 1995 was greater than nor-

Table 1. European corn borer damage and grain moisture of standard, Bt, and isoline corn hybrids during 1995 at Arlington, Hancock, and Lancaster, WI.

Corn hybrid	European corn borer treatment	Guthrie rating†	Number/plant		Grain moisture	
			Cavities	Live larvae	Stalk lodging	%
Standard	Inoculation	4.5	5.2	0.7	6.3	17.7
	Natural	2.0	--	--	6.7	18.6
Bt	Inoculation	1.1	0.4	0.1	2.6	18.9
	Natural	1.1	--	--	2.3	18.9
Isoline	Inoculation	4.0	2.8	0.4	4.8	18.3
	Natural	1.6	--	--	4.8	18.4
LSD(0.05)		0.4	0.7	0.2	1.7	0.8

† Guthrie rating 1 = No feeding, 9 = Most leaves with lesions longer than 1 in.

mal, and no insecticide treatment completely controlled ECBs throughout the season, we could not estimate how standard and isoline hybrids might yield in the absence of ECB.

In 1996, an additional insecticide treatment was added to give three levels of ECB infestation. Less stalk breakage was observed with Bt hybrids than with standard hybrids (Table 2). Also, spraying with permethrin decreased stalk breakage. Grain moisture of the Bt hybrids was not significantly different from the standard hybrids. Bt hybrids yields were similar regardless of ECB infestation (Fig. 2). Under natural infestation, yield of Bt hybrids was similar to standard hybrids. Inoculation of standard hybrids with ECB tended to decrease yield slightly. In the absence of ECB, yield of standard hybrids was 12 bu/acre greater than Bt hybrids.

Our results indicate that the initial Bt hybrid introductions yielded less than elite adapted hybrids. Breeding programs will most likely improve yield as Bt genes are incorporated earlier into the breeding cycles of elite inbreds.

Hybrid selection by farmers should be based on hybrid yield over an area of wide adaptation and management practices, and on the probability of significant ECB infestation. Bt hybrids will not yield best or be economically feasible in all environments.

Widespread use of Bt corn could lead to Bt resistance in the ECB. Development of resistance is favored because corn is the predominant host of the ECB. There is essentially no escape from Bt, since all feeding stages of the ECB occur on corn. ECB can produce multiple generations per year, which usually means faster expression of resistance. Resistance on

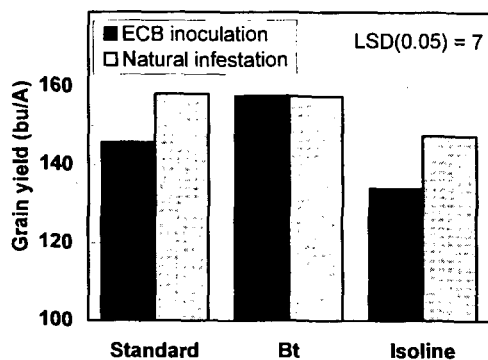


Fig. 1. Grain yield of standard, Bt, and isoline corn hybrids infested with European corn borer (ECB) during 1995 at Arlington, Hancock, and Lancaster, WI.

Table 2. European corn borer damage and grain moisture of standard and Bt corn hybrids during 1996 at Arlington, WI.

Corn hybrid	European corn borer treatment	Guthrie rating†	Number/plant		Stalk lodging	Grain moisture
			Cavities	Live larvae		
Standard	Inoculation	2.6	3.4	0.8	11.5	24.6
	Natural	1.7	2.7	0.5	7.8	25.4
	Insecticide	1.1	0.3	0.0	3.1	25.3
Bt	Inoculation	1.0	0.4	0.1	3.3	25.4
	Natural	1.0	0.1	0.1	2.7	25.2
	Insecticide	1.0	0.1	0.0	1.8	25.8
LSD(0.05)		0.1	0.4	0.2	2.1	NS

† Guthrie rating 1 = No feeding, 9 = Most leaves with lesions longer than 1 in.

a local scale probably will evolve because of a tendency for limited dispersal of the ECB moths (Mason et al., 1996). Derrick and Showers (1990) found that egg masses in fields are dependent upon moths present in nearby grass vegetation sites and that moths probably will use that field for oviposition. Development of Bt resistance has already been seen in the Indian meal moth (*Plodia interpunctella*) and ECB has demonstrated the ability to respond rapidly to intense laboratory selection infestation with commercial formulations of Bt (Huang et al., 1997).

The current cost per acre for Bt hybrid seed is equivalent to the cost of applying an insecticide, however, growers have to consider the long-term potential for economic infestations of ECB. Since standard non-Bt hybrids yielded as well as or better than Bt hybrids in the absence of infestation, Bt seed may not be worth the additional cost in areas where there is a low probability of infestation. For example, Wisconsin fall abundance surveys of ECB indicated that the 10-yr average populations for the northwest, northeast, and north central cropping districts averaged only 0.2 larvae/plant (WDATCP, 1995). Most loss calculations assume 5% loss/borer per plant (Boerboom et al., 1995). This indicates the three regions experience only about 1% annual yield loss from second generation ECB. Additionally, fall populations indicate low potential for first generation injury the following spring. Therefore, such regions will not experience appreciable returns from investing in Bt hybrids.

Planting individual fields with standard non-Bt hybrids to serve as "refugia" from selection pressure may delay development of resistance (Alstad and Andow, 1995). The spring flight of moths is attracted to the tallest corn (Wedberg, 1996). Planting Bt hybrids first and then switching to nontransgenic hybrids as the planting season pro-

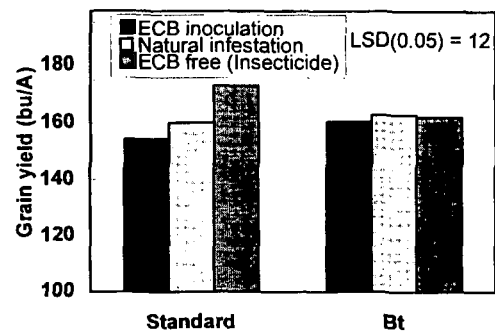


Fig. 2. Grain yield of standard and Bt corn hybrids infested with European corn borer (ECB) during 1996 at Arlington, WI.

gresses could provide a “halo” effect that reduces injury in the nontransgenic hybrids (Alstad and Andow, 1995) while reducing selection pressure and delaying development of resistance.

Development of resistance to Bt may be delayed because untreated areas can provide a source of susceptible moths to dilute the buildup of Bt resistant genes in the ECB population (McGaughey, 1985). The ECB can live on more than 200 species of plants, so alternatives to corn as a host exist. Not every field of corn will be planted to Bt corn so geographical mosaics of Bt and conventional hybrids will occur. Toxins vary among seed companies and hybrids; thus more than one toxin could be produced in a hybrid. Toxins could be used in conjunction with other forms of resistance developed by plant breeders such as DIMBOA and stalk strength.

Maintaining the effectiveness of Bt corn after it reaches the marketplace will be the responsibility of seed companies, educators, and farmers. Education about the limitations of this new technology and the potential development of insect resistance must be communicated to farmers along with management recommendations to insure that Bt technology is successful in the long run.

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