

Evaluation of Transgenic Bt Corn for Resistance to the Asian Corn Borer (Lepidoptera: Pyralidae)

KANGLAI HE, ZHENYING WANG, DARONG ZHOU, LIPING WEN, YANYING SONG, AND
ZHUYUN YAO¹

Institute of Plant Protection, Chinese Academy of Agricultural Sciences, No. 2 West Yuanmingyuan Road,
Beijing 100094, People's Republic of China

J. Econ. Entomol. 96(3): 935–940 (2003)

ABSTRACT The Asian corn borer, *Ostrinia furnacalis* (Guenée), is the most important insect pest on corn in China. Bt transgenic corn provides a new tool for Asian corn borer control. Monsanto's YieldGard Bt transgenic corn expressing Cry1Ab protein, and a non-Bt control, were evaluated in Beijing. Laboratory bioassays were carried out by exposing neonates to an agar-free diet containing Bt corn whorl leaves, tassels, and anthers, or by exposing neonates directly to fresh silk and pollen. These are the tissues initially attacked by neonates in the field. All of these tissues, with the exception of pollen, contained sufficient insecticidal protein to kill $\geq 95\%$ of larvae within 7 d. Surviving larvae had also not grown beyond first instar and weighed ≤ 0.1 mg. Although larvae feeding on Bt corn pollen were significantly smaller than those on non-Bt corn pollen, there was no significant difference in mortality. Field trials were also conducted with artificial infestations of Asian corn borer at mid whorl, late whorl, and silking stages. Damage ratings and number of larvae surviving per plant indicated that Bt corn was highly resistant to Asian corn borer. Therefore, YieldGard offers the potential for season-long protection against first- and second-generation Asian corn borer.

KEY WORDS Bt corn, transgenic plants, host plant resistance, *Ostrinia furnacalis*

CORN, *Zea mays* L., in China is annually attacked by the Asian corn borer (ACB), *Ostrinia furnacalis* (Guenée). Despite consistent losses estimated at 6–9 million tons for an ordinary year, farmers do not aggressively manage Asian corn borer by implementing traditional integrated pest management tactics such as biological control agents, light traps, and insecticide applications. More than 80% of the corn acreage is freely open to Asian corn borer. This attitude of neglect stems historically from several limitations of these tactics such as difficulties of application, the cost and skill demanded, biosafety and environmental concerns, and uncertainty about the benefits of the management. Obviously, favorable management options should be inexpensive, have a high and stable controlling effect, be simple and easy to apply, and not pollute. Cultural practices (manipulating cropping system, tillage practices), biological control (*Bacillus thuringiensis* (Bt), *Beauveria bassiana*, *Trichogramma* parasitoid wasps, and nematodes), and breeding for corn resistance, offer potential management solutions (Zhou et al. 1995). Of these, host plant resistance is an important component of ACB integrated management because the use of resistant hybrids derived through conventional plant breeding offers an economic, sta-

ble and ecologically sound approach to minimizing losses inflicted by ACB. Globally, during the last four decades, large investments and long-term research efforts have been put into plant breeding to develop pest resistant varieties and hybrids. Various conventional breeding techniques have facilitated significant improvements in resistance to corn borers (CIMMYT 1989, Mihm 1997). More recently, the development of transgenic plants (e.g., Bt corn) has introduced a new dimension to host plant resistance to pests.

The first genetically engineered corn, Naturgard and Maximizer (Event 176), containing a Bt gene and delivering an insecticidal protein, were registered in 1995 by the United States Environment Protection Agency (EPA) for Mycogen Corp. and Ciba Seeds (Anderson 1999). Bt field corn hybrids were firstly commercially available to farmers in 1996. Subsequently, MON810 and Bt-11 were registered by EPA as YieldGard for Monsanto and Northrup King in 1996. In addition, Dekalb and AgrEvo/PGS received registrations for Bt-Xtra (DBT-418) and StarLink (CBH-351) in 1997 and 1998, respectively. Among these events, Event 176, MON810, and Bt-11 express Cry1Ab; DBT-418 expresses Cry1Ac; and CBH-351 expresses a Cry9C protein. These events rely on constitutive promoters to achieve Bt protein expression in all of the tissues that need to be protected against damage from lepidopteran herbivores. All of the

¹ Monsanto Company, 800 North Lindbergh Blvd., St. Louis, MO 63167.

events other than Event 176 contain the cauliflower mosaic virus ^{35}S promoter. Event 176 contains two promoters, one to obtain expression in green tissues and the other to ensure high Bt expression in pollen (Koziel et al. 1993, Estruch et al. 1994). EPA extends registration of YieldGard (MON810 and Bt-11) expressing Cry1Ab and Herculex (TC1507) expressing Cry1 F Bt corn in 2001 (U.S. EPA 2001). These first-generation Bt corn hybrids were initially developed for control of the European corn borer, *O. nubilalis* (Hübner), but have the potential to control other corn borers. In field tests against natural and supplemented European corn borer infestations, Bt corn (regardless of event) provide more than 99% control of first-generation European corn borer larvae in whorl-stage corn. However, control of late-season corn borer infestations differs markedly among Bt events. The MON810 and Bt11 provide 93% control of southwestern corn borer, *Diatraea grandiosella* Dyar, whereas Event 176 afforded only 19% control (Koziel et al. 1993; Armstrong et al. 1995; Williams et al. 1997, 1998; Jansens et al. 1997; Ostlie et al. 1997; Archer et al. 2000, 2001).

Because the efficacy of Bt transgenic corn YieldGard against ACB has not been studied, we initiated this research to evaluate the efficacy and potential for Bt transgenic corn to control ACB in China. In this paper, we present data demonstrating that YieldGard is highly resistant to ACB.

Materials and Methods

Plants and Insects. Monsanto's YieldGard Bt transgenic corn (event MON810) expressing Cry1Ab protein, and a non-Bt control, were grown at the experimental farm of the Institute of Plant Protection, Chinese Academy of Agricultural Sciences in Beijing. Because the flowering dates were not synchronized between the Bt corn and the non-Bt control, another advanced line (MC37) was used as the non-Bt control in the pollen and anther tests.

Asian corn borer egg masses and neonates used in this study were obtained from a laboratory colony that originated from a Hengshui population and had been maintained on an agar-free semiartificial diet of ACB (Song et al. 1999) for several generations.

Laboratory Bioassays. Resistance of Bt transgenic corn to ACB was evaluated with whorl leaves, immature tassels, and anthers using a modification of the technique for resistance evaluation of corn to European corn borer in the laboratory described by Zhou et al. (1984). The agar-free semiartificial diet of ACB was adapted for the bioassay. All solid ingredients of the diet were blended and finely ground with a kitchen grinder. Sixty grams of test tissues (whorl leaves and tassels of Bt corn or non-Bt control plants) were cut into small pieces with a pair of scissors. Each sample then was processed in the grinder with 60 ml of distilled water. The resulting juice was poured into a flask that contained 60 g of processed solid ingredients of the diet to form the testing medium. Although concentrations of Bt may vary in field situations,

MON810 averages $9.35\text{ }\mu\text{g/g}$ (fresh weight) in leaves and $3.65\text{--}4.65\text{ }\mu\text{g/g}$ (f.w.) in the whole plant (AGBIOS 2001).

Glass vials (8.0 cm high $\times$ 2.5 cm diameter) with rubber stoppers were used as rearing containers. A hole 0.5 cm in diameter was made in the stopper and sealed with a piece of stainless steel screen. Each container was filled with $\approx 6\text{ g}$ of testing medium, infested with 10 neonate larvae of ACB, and served as a replicate. Twenty (mid-whorl leaves) or thirty (last two leaves and young tassel, respectively) containers were made for each of the Bt corn and non-Bt control. All containers were moved to a rearing room at 70–80% RH, 28–30°C, and a photoperiod of 16:8 h (L:D). Survivors and larval weights were recorded after 7 d. Larvae that had not grown beyond first instar and weighed $\leq 0.1\text{ mg}$ were considered to be dead.

Bundles of fresh silks from twenty plants were collected from Bt corn plants and non-Bt control plants at silking stage in the field. Each bundle of silks was placed into a plastic container (10.5 cm high $\times$ 11.5 cm diameter) with a disc of wet filter paper at the bottom. Two blackhead ACB egg masses ($\approx 20\text{--}30$ eggs) were placed into each container. The same incubation conditions were used as described above. Number of survivors and weights were recorded 6 d later.

To collect pollen, pollen-shedding tassels were covered with pollination bags. In the late morning of the next day, pollen and anthers were collected by shaking the tassel bags and then a screen was used to separate the pollen from anthers. The anthers were fast frozen with liquid nitrogen and ground with a mortar and pestle. The resulting powder was mixed with processed diet (1:1) and distilled water to get the testing medium. Other procedures were the same as for testing of the whorl leaves. Fresh pollen was used as the test diet. Expression of Bt protein can be as low as $0.09\text{ }\mu\text{g/g}$ (fresh weight) in pollen (AGBIOS 2001). Each container contained 400 mg of the pollen. It then was infested with five neonate larvae and transferred to a rearing room. Twenty replicate containers were created for each of the Bt corn and non-Bt control, respectively. Survivors and larval weights were recorded 3 d later. Because the flowering dates were not synchronized between the Bt corn and its non-Bt control, an advance line MC37 was used as a non-Bt Check in the anther and pollen bioassays.

Field Evaluations. Effectiveness of first-generation (leaf-feeding resistance, tassel resistance) and second-generation (silk resistance) control of ACB with Bt transgenic corn was evaluated in the field with traditional artificial infestation techniques similar to that described by He et al. (2000a). Trial plots contained two 5-m rows with three replications.

Leaf-feeding Resistance. All test plants in the two 5-m rows were artificially infested with two blackhead ACB egg masses ($\approx 40\text{--}60$ eggs) at mid-whorl stage. Leaf-feeding ratings (on a 1–9 scale, in which 1 = the most resistant and 9 = the most susceptible) (He et al. 2000a) and borer density were estimated within 12 d.

Tassel Resistance. All test plants in the two 5-m rows, different from those used for leaf-feeding resis-

Table 1. Mortality (mean $\pm$ SEM) of Asian corn borer larvae feeding on diet incorporated with Bt and non-Bt maize tissues at vegetative stages

Variety	Larval mortality (%)		
	Mid-whorl leaves	Last 2 leaves at V17	Young tassel at V17
Bt maize	100 $\pm$ 0 ^a	100 $\pm$ 0 ^a	100 $\pm$ 0 ^a
Non-Bt control	24.00 $\pm$ 3.36 ^a	12.67 $\pm$ 2.39 ^a	16.33 $\pm$ 2.17 ^a

^a The means within a column were significantly different ($P < 0.05$) by *t*-test.

tance evaluation, were artificially infested at vegetative stage V17 (17 leaf stage). Two blackhead ACB egg masses were placed upon the young tassel. Subsequently, the whole tassel with its last enveloping whorl leaves was covered with a pollination bag, which could protect the larvae from predators. Damage ratings (on a 1–5 scale, in which 1 = the most resistant and 5 = the most susceptible) (He et al. 2000a) and larval numbers were recorded 12 d later.

Silk Resistance. At VT (tasseling) stage, the ears (before silking) in the two 5-m rows, different from those used for leaf-feeding and tassel resistance evaluation, were covered with ear-bags to prevent pollination. When plants developed to full green silk stage, ears were carefully self or full sib pollinated and infested with 20–30 neonate larvae per silk mass with a Bazooka applicator (CIMMYT 1989). The ear-bags were then replaced and the bag mouths were fastened with rubber bands to protect the larvae from predators. Damage ratings (revised centimeter scale of Widstrom 1967) and larvae densities were estimated after 15 d.

Experimental Design and Data Analysis. Laboratory and field assays used completely randomized designs. Data were analyzed statistically using the PROC TEST procedure (SAS Institute 1989). Analyses included damage ratings, larvae mortality, larval weight, and the number of larvae in stalks for mid-whorl, V17, and silk infestations.

Results

Laboratory Bioassays. In the laboratory bioassays, $\geq 99.5\%$ of the larvae were killed within 7 d when they fed on diet containing Bt corn tissue of mid-whorl leaves, last two leaves, and young tassel at V17. Only one small larva survived among 200 larvae feeding on diet containing the mid-whorl leaves. This larva was a first instar and weighed ≤ 0.1 mg, and so was considered effectively dead. In contrast, larval mortality was only 12.7–24.0% when feeding on diet containing

non-Bt control tissue. The surviving larvae were late third to early fourth instar and weighed 12.2–19.8 mg/larva. These results indicate that Bt corn tissues at vegetative stages contains sufficient Bt protein to kill all neonate larvae of ACB ($t = 7.1512$, $df = 38$, $P = 0.0001$, for mid-whorl leaves; $t = 5.2907$, $df = 58$, $P = 0.0001$, for last two leaves at V17; $t = 7.5270$, $df = 58$, $P = 0.0001$, for young tassel at V17) (Table 1).

Fresh silk bioassays showed that no ACB larvae survived on Bt corn silks, but an average of 34 larvae survived on non-Bt control silks. The Bt corn silks were still in good condition after the 6 d test period. However, non-Bt control silks were completely consumed by the larvae. The surviving larvae were at third instar. This result indicates that Bt corn silk contains adequate Bt protein to kill neonate ACB larvae.

Larvae fed on the diet containing Bt corn anthers also suffered 100% mortality. The difference between the Bt corn and non-Bt corn MC37 was dramatic ($t = 16.8475$, $df = 18$, $P = 0.0001$). The latter only caused 16% mortality (Table 2). This indicates that Bt corn anthers contain sufficient Bt protein to kill any neonate ACB larvae.

However, mortality was not significantly different for neonate larvae fed on Bt corn pollen compared with non-Bt corn MC37 within 3 d ($t = 1.2274$, $df = 38$, $P = 0.2272$), though the weight of larvae feeding on Bt corn pollen was significantly lower than those on the non-Bt check MC37 ($t = 6.6606$, $df = 38$, $P = 0.0001$) (Table 2). The average weight of larvae that fed on the pollen of MC37 was increased to 0.513 mg/larva from 0.1 mg/larva. This increase was almost as those fed on the artificial diet. It could indicate that pollen of MC37 is not toxic to ACB. These results suggest that Bt protein is expressed in the pollen of Bt corn, but not at a sufficient level to cause significant mortality of Asian corn borer larvae.

Field Evaluations. Field tests showed that minimal damage was seen for leaf feeding, tassel feeding, and silk feeding on Bt corn under artificial infestation with ACB at mid-whorl, V17, and silk stages. There were no

Table 2. Mortality and weight (mean $\pm$ SEM) of Asian corn borer larvae feeding on diet containing Bt and non-Bt maize anthers and pollens

Variety	Mortality %		Larval weight, mg/larva
	Anther	Pollen	Pollen
Bt maize	100 $\pm$ 0 ^a	9 $\pm$ 3.069 ^b	0.298 $\pm$ 0.017 ^a
MC37 (non-Bt)	16.0 $\pm$ 4.989 ^a	15.0 $\pm$ 3.804 ^b	0.513 $\pm$ 0.027 ^a

^a The means within a column were significantly different ($P < 0.05$) by *t*-test.

^b The means within a column were not significantly different ($P \geq 0.05$) by *t*-test.

Table 3. Damage ratings and larval numbers (mean $\pm$ SEM) for Bt maize and a non-Bt control evaluated in the field under artificial infestation with Asian corn borer at mid-whorl, V17, and silk stages

Variety	Mid-whorl		Tassel		Silk	
	Leaf feeding ratings	Larvae surviving/plant	Tassel damage ratings	Larvae surviving/plant	Ear damage ratings	Larvae surviving/plant
Bt maize	1 $\pm$ 0 ^a	0 $\pm$ 0 ^a	1 $\pm$ 0 ^a	0 $\pm$ 0 ^a	0 $\pm$ 0 ^a	0.19 $\pm$ 0.19 ^a
Non-Bt control	7.44 $\pm$ 0.53 ^a	5.88 $\pm$ 0.65 ^a	4.46 $\pm$ 0.23 ^a	14.04 $\pm$ 1.49 ^a	7 $\pm$ 0.339 ^a	10.13 $\pm$ 1.62 ^a

^a The means within a column were significantly different ($P < 0.05$) by *t*-test.

larvae surviving in mid-whorl leaves and tassels. Only one ear had four small larvae beyond second instar surviving on the silk. In contrast, significant leaf feeding, tassel feeding, and silk feeding were observed in non-Bt control plots ($t = 18.2270$, $df = 30$, $P = 0.0001$; $t = 15.0318$, $df = 50$, $P = 0.0001$; $t = 20.6460$, $df = 58$, $P = 0.0001$, respectively). On average, >5 larvae were found per plant (Table 3) ($t = 9.0229$, $df = 30$, $P = 0.0001$; $t = 9.4026$, $df = 50$, $P = 0.0001$; $t = 6.0926$, $df = 30$, $P = 0.0001$), and these larvae were at fifth instar. These results indicate that Bt corn is highly resistant to ACB in the mid-whorl, V17, and silk stages.

Discussion

Unlike the chemical controls used to kill corn borer larvae, Bt transgenic corn affects larvae through ingestion of the Bt protein. Therefore, it is important to know the efficacy of the plant tissues in which neonate larvae initially feed. Both first-generation neonate larvae of European corn borer and ACB tend to establish on whorl leaves (Zhou et al. 1995, Mason et al. 1996). Thus first-generation corn borer resistance requires leaf-feeding resistance. Average protein expression, as measured in samples obtained from field trials at six locations, was 9.35 $\mu\text{g/g}$ (fresh weight) in leaves of event MON810, and ranged from 3.65 to 4.65 $\mu\text{g/g}$ (f.w.) in the whole plant (AGBIOS 2001). Leaf disk assays have shown that Bt corn expresses adequate Bt protein to kill any European corn borer neonate larvae (Kozziel et al. 1993). The same results were obtained with Bt corn leaves and ACB in this research.

Tassel resistance evaluations have never been reported. This probably is because tassel resistance has never been considered important for European corn borer. However, this is not the case for ACB in China. Tassel resistance plays an importance role in ACB control (He et al. 2000b). Cold tolerant and high yielding long-season hybrids are used with early plantings in the spring corn region. The first-generation Asian corn borer eggs are deposited on corn plants during the late whorl stage (V17 stage). In the summer corn region, corn is planted early with preharvest (winter wheat) interplantings or immediately after the harvest of wheat. Moreover, first-generation ACB eggs are always overlapping with second-generation. Eggs of the end of first generation and the beginning of second generation are deposited on corn plants during the late-whorl stage. Field corn infested at late-whorl stage by ACB suffers significantly more loss than that infested at mid-whorl and early silking stages

(Cheu et al. 1964). This is because neonate larvae will directly attack the immature tassels, which will result in a much higher survival rate of larvae, ≈ 20 –25 fold, than that on whorl leaves. Therefore, tassel resistance is of utmost important for ACB control in China. Bt corn tassels proved to have adequate expression of Bt protein to kill ACB neonates.

Second-generation European corn borer become established on pollen accumulated in the axils of leaves, on sheath-collar tissue, and on ear shoots, husks, and silks (Guthrie et al. 1969, 1970). European corn borer larvae prefer to feed on sheath-collar tissue instead of on husk-silk tissue. The primary sites of resistance to second-generation larvae are sheath-collar tissue (Guthrie et al. 1980). Hybrids with the MON810 event proved to be adequate to control second-generation European corn borer infested as neonate larvae in the axil of the ear leaf at anthesis (Archer et al. 2000, 2001). For second-generation ACB, fresh silk is the most preferred tissue for neonate larvae, resulting in a very high survival rate. Second-generation ACB resistance is actually silk feeding resistance (He et al. 2000b). Results from this study indicate that fresh silks of Bt corn express sufficient Bt protein to be protected from larval feeding.

Pollen accumulated in the axils of leaves comprises a portion of the neonate diet of second-generation ACB during first and second instar. Therefore, adequate expression of Bt protein in pollen may be important for control of second-generation ACB. Although the weight of larvae that fed on the pollen of Bt corn was lower than those on non-Bt corn MC37, the expression of Bt protein in the pollen was not adequate to cause significantly mortality of neonate larvae. This finding is consistent with a report from Agriculture and Biotechnology Strategies (AGBIOS) Inc. The expression of protein can be as low as 0.09 $\mu\text{g/g}$ (fresh weight) in pollen (AGBIOS 2001). The low expression of Bt protein in pollen might lead to selection of Bt resistant populations of ACB, though the results of the field evaluations suggest that low protein expression in pollen is not sufficient to enable larvae to survive on Bt plants. This concern needs to be further examined.

Monsanto Bt transgenic corn hybrids with the MON810 event provide good control of first- (leaf-feeding damage) and second-generation (stalk, shank, and ear damage) European corn borer in mid-whorl and tassel growth stages under field conditions (Kozziel et al. 1993; Armstrong et al. 1995; Archer et al. 2000, 2001). Data presented in this paper demonstrate the

effectiveness of Bt transgenic corn YieldGard with the MON810 event for controlling first- (in the mid-whorl and V17 stages) and second-generation Asian corn borer (silk stage). Near immunity to neonate larvae of Bt corn can provide an excellent way to control ACB, and offers potential for season-long protection against first- and second-generation ACB. This research indicated that Bt transgenic corn presents us a new management tactic for improving our current integrated pest management program for Asian corn borer in China and possibly other Asian countries.

Acknowledgments

We thank Xinzhi Ni, Department of Entomology, Montana State University; Graham Head, Monsanto LLC, St. Louis; and Jianzhou Zhao, Department of Entomology, Cornell University, for critical review of an earlier version of the manuscript. This research was supported in part by National Natural Science Foundation of China (NSFC) grant # 39970489 and by Monsanto.

References Cited

- [AGBIOS] Agriculture and Biotechnology Strategies Incorporated. 2001. Crop database, MON810. Essential Biosafety. (<http://www.essentialbiosafety.info/dbase.php>)
- Anderson, J. 1999. Proceedings of EPA/U.S. Dep. Agric. Workshop on Bt Crop Resistance Management, June 18, 1999, Chicago IL.
- Archer, T. L., G. Schuster, C. Patrick, G. Cronholm, E. D. Bynum, Jr., and W. P. Morrison. 2000. Whorl and stalk damage by European and Southwestern corn borers to four events of *Bacillus thuringiensis* transgenic maize. *Crop Prot.* 19: 181–190.
- Archer, T. L., C. Patrick, G. Schuster, G. Cronholm, E. D. Bynum, Jr., and W. P. Morrison. 2001. Ear and shank damage by corn borers and corn earworms to four events of *Bacillus thuringiensis* transgenic maize. *Crop Prot.* 20: 139–144.
- Armstrong, C. L., G. B. Parker, J. C. Pershing, S. M. Brown, P. R. Sanders, D. R. Duncan, T. Stone, D. A. Dean, D. L. Deboer, J. Hart, A. R. Howe, F. M. Morrish, M. E. Pleau, W. L. Petersen, B. J. Reich, R. Rodriguez, C. G. Santino, S. J. Sato, W. Schuler, S. R. Sims, S. Stehling, L. J. Tarochione, and M. E. Fromm. 1995. Field evaluation of European corn borer control in progeny of 173 transgenic corn events expressing an insecticidal protein from *Bacillus thuringiensis*. *Crop Sci.* 35: 550–557.
- Cheu, S. P., D. R. Chow, H. F. Tung, and C. H. Lee. 1964. Yield reduction of corn caused by the European corn borer at different development stages. *Acta Phytolacica Sinica* 3(3): 307–312.
- [CIMMYT] Centro Internacional de Mejoramiento de Maiz y Trigo. 1989. Toward insect resistant maize for the third world: Proceedings of the International Symposium on Methodologies for Developing Host Plant Resistance to Maize Insect. CIMMYT, Mexico, D. F., Mexico.
- Estruch, J. J., S. Kadwell, E. Merlin, and L. Crossland. 1994. Cloning and characterization of a maize pollen specific calcium-dependent protein kinase. *Proc Natl Acad Sci USA* 91: 8837–8841.
- Guthrie, W. D., J. L. Huggans, and S. M. Chatterji. 1969. Influence of corn pollen on the survival and development of second-brood larvae of the European corn borer. *Iowa State J. Sci.* 44: 185–192.
- Guthrie, W. D., J. L. Huggans, and S. M. Chatterji. 1970. Sheath and collar feeding resistance to the second-brood European corn borer in six inbred lines of dent corn. *Iowa State J. Sci.* 44: 297–311.
- Guthrie, W. D., F. A. Onukogu, W. H. Awadallah, and J. C. Robbins. 1980. European corn borer: evaluation of resistance in husk-silk tissue of inbred lines of corn. *J. Econ. Entomol.* 73: 178–180.
- He, K. L., Z. Y. Wang, D. R. Zhou, L. P. Wen, and Y. Y. Song. 2000a. Methodologies and criteria for evaluating maize resistance to the Asian corn borer. *J. Shenyang Agri. Univ.* 31: 439–443.
- He, K. L., D. R. Zhou, and Z. Y. Wang. 2000b. Progress of maize resistance research to the Asian corn borer in China. In China Association of Agricultural Science Societies [eds], Prospects of maize genetics and breeding for the 21st century. Proceedings of an International Maize Genetics and Breeding Symposium, 15–17 September 2000, Changchun, China.
- Jansens, S., A. van Vliet, C. Dickburt, L. Buysse, C. Piens, B. Saey, A. D. Wulf, V. Gosselé, A. Paez, E. Göbel, and M. Peferoen. 1997. Transgenic corn expressing a Cry9C insecticidal protein from *Bacillus thuringiensis* protected from European corn borer damage. *Crop Sci.* 37: 1616–1624.
- Kozziel, M. G., G. L. Beland, C. Brown, N. B. Carozzi, R. Crenshaw, L. Crossland, J. Dawson, N. Desai, M. Hill, S. Kadwell, K. Launis, K. Lewis, D. Maddox, K. McPherson, M. R. Meghji, E. Merlin, R. Rhodes, G. W. Warren, M. Wright and S. V. Evola. 1993. Field performance of elite transgenic maize plants expressing an insecticidal protein derived from *Bacillus thuringiensis*. *Bio/Technology* 11: 194–200.
- Mason, C. E., M. E. Rice, D. D. Calvin, J. W. Van Duyn, W. B. Showers, W. D. Hutchison, J. F. Witkowski, R. A. Higgins, D. W. Onstad, and G. P. Dively. 1996. European corn borer, ecology and management. North Central Regional Extension Publication No. 327. Published by Iowa State University, Ames, Iowa.
- Mihm, J. A. [ed.]. 1997. Insect Resistant Maize: Recent Advances and Utilization; Proceedings of an International Symposium held at the International Maize and Wheat Improvement Center (CIMMYT) 27 November–3 December 1994. CIMMYT, Mexico, D. F., Mexico.
- Ostlie, K. R., W. D. Hutchison, and R. L. Hellmich. 1997. Bt corn and European corn borer: long-term success through resistance management. North Central Region Extension Publication 602. University of Minnesota, St. Paul, MN.
- SAS Institute. 1989. SAS/STAT user's guide, version 6, 4th ed. SAS Institute, Cary, NC.
- Song, Y. Y., D. R. Zhou, and K. L. He. 1999. Studies on mass rearing of Asian corn borer: development of a satisfactory non-agar semi-artificial diet and its use. *Acta Phytolacica Sinica* 26: 324–328.
- [EPA] U.S. Environmental Protection Agency. 2001. Biopesticides registration action document for *Bacillus thuringiensis* plant-incorporated protectants (October 16, 2001). U. S. Environmental Protection Agency (http://www.epa.gov/pesticides/biopesticides/reds/brad_bt_pip2.htm).
- Widstrom, N. W. 1967. An evaluation of methods for measuring corn earworm injury. *J. Econ. Entomol.* 60: 791–794.
- Williams, W. P., P. M. Buckley, J. B. Sagers, and J. A. Hanten. 1998. Evaluation of transgenic corn for resistance to corn earworm (Lepidoptera: Noctuidae), fall armyworm (Lepidoptera: Noctuidae) and southwestern corn borer

- (Lepidoptera: Crambidae) in a laboratory bioassay. *J. Agric. Entomol.* 15: 105–112.
- Williams, W. P., J. B. Sagers, J. A. Hanten, F. M. Davis, and P. M. Buckley.** 1997. Transgenic corn evaluated for resistance to fall armyworm and southwestern corn borer. *Crop. Sci.* 37: 957–962.
- Zhou, D. R., W. D. Guthrie, and C. C. Chen.** 1984. A bioassay technique for screening inbred lines of maize for resistance to leaf feeding by the European corn borer. *Maydica*. XXIX: 69–75.
- Zhou, D. R., K. L. He, Z. Y. Wang, Z. H. Ye, L. P. Wen, Y. X. Gao, and Y. Y. Song.** 1995. Asian corn borer and its integrated management. Golden Shield Press, Beijing, China.

Received for publication 1 October 2002; accepted 23 December 2002.
