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SURVIVAL AND PLANT DAMAGE ASSESSMENT OF ASIAN CORN BORER, *Ostrinia furnacalis* (Guenee), TO SINGLE TRAIT BT 11 MAIZE

Merdelyn T. Caasi-Lit[1,*], Victor V. Alpuerto[2], Mark Bryan T. Lontoc[1], Gian R. Pescadero[1], Rachel H. Dacuba[1], Jenifer P. Bigcas[1], Emmanuel V. Elladora[1] & Edwin A. Benigno[1,3]

[1]Entomology Laboratory, Institute of Plant Breeding, College of Agriculture and Food Science (CAFS), University of the Philippines Los Baños (UPLB), College, Laguna, 4031 Philippines; *Corresponding author: mclit@up.edu.ph
[2]Former Asia Pacific IRM Lead, Syngenta Philippines Inc. 12/F Two World Square, 22 Upper McKinley Road, McKinley Town Center, Fort Bonifacio 1630 Taguig City; Stewardship Director, Crop Life Asia, Unit 5E MAPFRE Asian Corporate Center, Acacia Avenue, Madrigal Business Park, Ayala Alabang, Muntinlupa City, Metro Manila
[3]Researcher and Statistician, University Research Center, New Era University, Quezon City and Consultant Statistician, Entomology Laboratory, Institute of Plant Breeding, CAFS, UPLB

ABSTRACT

Field studies were conducted to determine the efficacy of single trait Bt corn product (Bt11xGA21) against the Asian corn borer, *Ostrinia furnacalis* (Guenee). The studies were set up in two sites, namely: Barangay Carabatan Punta, Cauayan, Isabela and Barangay San Isidro, General Santos City, South Cotabato, Philippines, at vegetative and reproductive stages of corn, for two seasons (dry and wet), and with artificial and natural infestation of Asian corn borer. No Asian corn borer survived on Bt corn (Bt11xGA21) during both vegetative and reproductive stages in both trial locations while survival of Asian corn borer on non-Bt (GA21) corn was consistently higher during reproductive stage of corn in both sites. Therefore, Bt maize, Bt11xGA21, remains highly efficacious against the Asian corn borer up to the present, since its commercial launch in the Philippines, a decade back.

Key words: Asian corn borer, bioefficacy, Bt11 maize, *Ostrinia furnacalis*

INTRODUCTION

It is essential to develop a comprehensive insect resistance management (IRM) strategy to prolong the effectiveness of Bt crops and delay the evolution of pest resistance to Bt toxins. At present, a high dose/refuge strategy is considered one of the more effective IRM strategies for delaying resistance evolution to Bt toxins (Bates et al., 2005).

Bt11 is the second Bt corn event that was introduced in the Philippines in 2005 by Syngenta. The Bt11 corn contains a gene which encodes for the production of Cry1Ab protein. This insecticidal protein protects the plant from insect damage particularly the Asian corn borer (ACB), *Ostrinia furnacalis* (Guenee). Bt11 is stacked with GA21, an herbicide tolerant corn, and has been in the commercial market for 12 years. It is included in the Lists of Approval Registry prepared by the Department of Agriculture - Bureau of Plant Industry.

This study aimed to compare the survival of *O. furnacalis*, on Bt11xGA21 corn and non-Bt corn hybrid (GA21) and conduct plant damage assessments.

MATERIALS AND METHODS

Test Entries

Test materials are seeds of commercial Bt corn hybrid NK8840BtGT (Bt11xGA21) and non-Bt corn hybrid NK8840GT (GA21).

Test locations and ACB infestation

The field efficacy trials were conducted for two seasons (2015-2016 dry and wet seasons), in Barangay Carabatan Punta, Cauayan City, Isabela Province, in the northern part of Luzon, and in Crossing Conel Road, Barangay San Isidro, General Santos (GenSan) City, South Cotabato Province, Mindanao, Philippines.

Initial populations of the ACB were collected near the trial sites and mass reared at the Entomology Laboratory, Institute of Plant Breeding, University of the Philippines Los Baños, College, Laguna for Isabela populations and at the Insect Rearing Laboratory of Syngenta, General Santos City, following the procedures of Caasi-Lit et al. (2012). Second instars were prepared as test insects for field infestation in both locations.

ACB infestation was done at the vegetative (30 days after planting, DAP) and reproductive (40 DAP) stages of corn. A new infestation technique, "corn stalk barbecue technique" was developed and adopted in this study (Caasi-Lit et al. 2017). It was found very efficient in delivering the second instar 4-day old larvae onto the whorl as shown by the uniform damage observed at the vegetative and reproductive corn stages, five and 10 days after infestation (DAI). This new technique was found to be useful for field bioefficacy testing of Bt and non-Bt corn under both greenhouse and field conditions.

Experimental design, treatments and field lay-out

The study was laid out in a randomized complete block design (RCBD) with two treatments in three replications consisting of 36 plots divided into vegetative and reproductive stages, and artificial and natural infestation.

Each replication plot had seven 5.0-m long rows. Plant spacing was 0.70 m between rows and 0.20 m between hills, with a total of 24 plants per row. Two outer rows (Rows 1 & 7) served as borders. Fifty 4-day old second instar larvae were artificially infested per plant. This was done twice in separate set ups: first at the vegetative stage, 30 DAP, and second at the reproductive stage, 40 DAP. Data were gathered from 100 plants per plot in the five inner rows (2-6) with 20 plants per row (plants 3-22).

Data Collection

Five and 10 DAI at vegetative and reproductive stages, all infested plants in each treatment were examined for leaf feeding damage. Damage caused by ACB was assessed following a harmonized rating scale by Guthrie (1960) (Table 1). The rating scale developed by Wiseman et al. (1966) served as reference only for detailed damage description.

Fifteen DAI at vegetative and reproductive stages set-up, destructive sampling was done to record the larval and pupal survival. Plants in each plot were carefully dissected and examined for the presence of borer holes and stalk damage. Borer holes were counted and recorded per plant in each treatment. For the reproductive stage infestation, all plants that showed tassel and ear injury and stalk tunneling were carefully examined and counted. Plants were also dissected carefully to record and count the number of individual feeding galleries. After dissection, tunnel lengths were measured. Larval and pupal survival were recorded together with ear and stalk damage assessment.

Statistical Analysis

Data on the number of recovered larvae were log transformed and analyzed using Minitab version 12 while those for damage rating were analyzed using Repeated Measures ANOVA (SPSS version 21). The number of borer holes and tunnel length were presented as means with standard errors. Separation of means was computed using Tukey's test.

RESULTS AND DISCUSSION

Corn Borer Damage

The field bioefficacy studies showed that Bt plants were resistant and the non-Bt plants (GA21), susceptible to second instar ACB larvae. The damage to the Bt11xGA21 and GA21 hybrids were consistent using artificial infestation of ACB larvae.

At five DAI, during the vegetative stage, damage on Bt plants was characterized by tiny rasps or faint spots (Figure 1a), suggesting that larvae initially attempted to feed on the upper epidermis down to the spongy cells at the lower epidermis, leaving the lower cuticle. The lower cuticle dried up and later formed few pinholes at 10 DAI.

Damage on non-Bt plants was very visible, with larvae initially scraping the leaves then forming pinholes and later match head-sized holes (Figure 1b). With continuous feeding, several lesions were formed with the dried frass scattered around the damaged portion. Almost all leaves during the vegetative stage were damaged and the whorls were full of holes and lesions. During the reproductive stage, no damage or only tiny rasps were seen on Bt plants at five DAI. For non-Bt plants, the remaining whorl leaves were heavily damaged with some of the larvae feeding on the tassel. Damage was more pronounced at 10 DAI with visible feeding and boring on collar and leaf sheaths. Larvae were already observed boring into the soft portion of the nodes above and below the ears. Larvae that had eaten some leaf portions of the Bt plants became sluggish and

Table 1. Rating scale for leaf-feeding damage for vegetative and reproductive stages at five and 10 days after infestation (Adopted from Guthrie, 1960 and Wiseman et. al., 1966).

Qualitative Description	Rating scale		Description
	Guthrie (1960)	Wiseman et al. (1966)	
Highly Resistant	1 – 2		No leaf damage on the plants, few pinholes about 0.5 mm in diameter
		0	No damage
		1	Few pinholes
Resistant	3-4		Light infestation, small shot holes on several leaves about 2 mm in diameter
		2	Several to many pinholes
		3	Few shot holes & 1 or 2 elongated lesions
Intermediate	5-6		Several leaves with elongated lesions about 2.5 cm in diameter
		4	Several shot holes & few elongated lesions
		5	Several shot holes & elongated lesions
Susceptible	7-8		Moderate to severe infestation, elongated lesions more than 2.5 cm in diameter
		6	Many shot holes, and several elongated lesions, few leaves eaten
		7	Several lesions, leaves are eaten away, leaves start drying & dying
		8	Many elongated lesions, leaves are eaten away, and drying very visible
Highly Susceptible	9-10		Severe infestation, all lesions merged, & leaves appeared shredded
		9	Whorl almost eaten, plenty of elongated lesions, leaves dry & dying
		10	Dying or dead plants

later stationary. After one or two days, the stiff larvae turned black with traces of dried body fluids underneath the integuments. They eventually died (Figure 1a).

Pooled Data per Plot for Damage Rating

Repeated measures ANOVA for pooled data per plot on damage ratings showed significant main effects between plant stages (vegetative vs. reproductive), infestation types (artificial vs. natural), and treatments (Bt vs. non-Bt) (Table 2). There were no significant effects between locations (Cauayan vs. GenSan), and seasons (wet vs. dry).

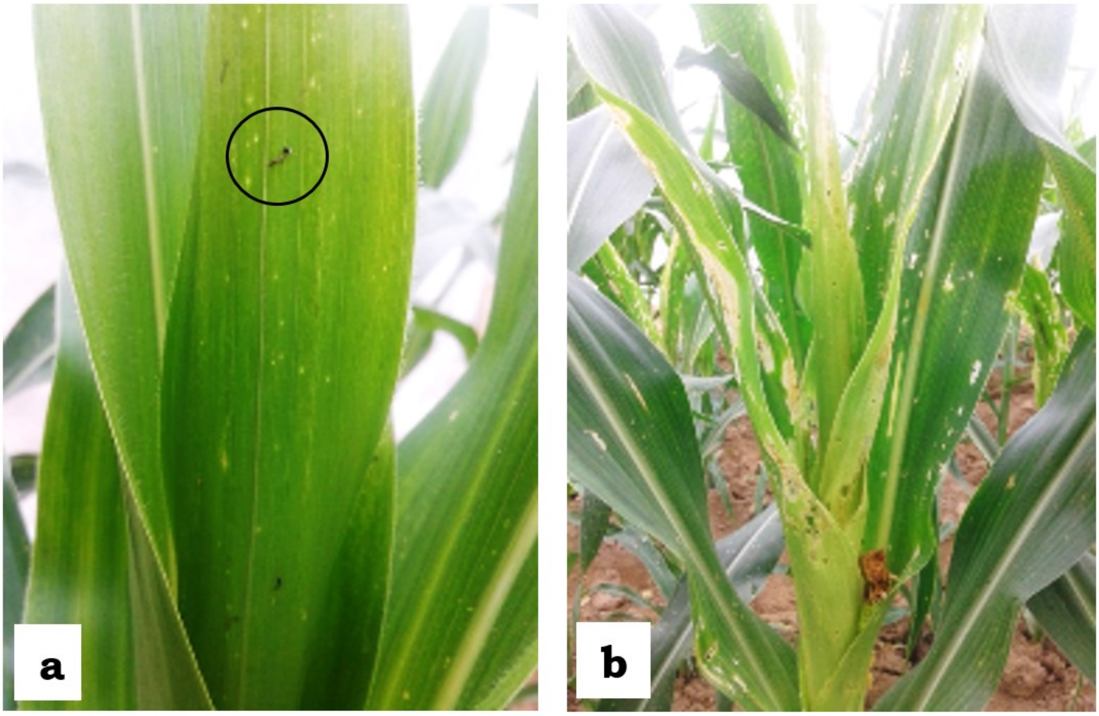

Figure 1. Initial feeding on corn plants. **a.** Bt plants showing faint spots and rasps by second instar five days after infestation at vegetative plots with dying larvae and dead black larvae after 1-2 days. **b.** non-Bt plants showing pinholes and match head-sized holes and damaged whorl.

Table 2. Analysis of variance for damage rating. Degree of freedom for all = 1.

Source of Variation	Sum of Squares	Mean Square	F	Significance
Intercept	713.44	713.44	2734.69	0.00*
Location	0.31	0.31	1.18	0.28ns
Season	0.00	0.00	0.02	0.90ns
Stage	25.89	25.89	99.25	0.00*
Infestation	86.63	86.63	332.05	0.00*
Treatment	121.01	121.01	463.84	0.00*

*Means main effect is significant at 0.05 level

Borer Holes and Tunnels

The damage on stalks of Bt and non-Bt plants differed significantly, with borer holes and tunnels recorded only on non-Bt plants than but none on the Bt plants (Table 3). The borer holes and tunnels were observed mostly during the first and second seasons at the reproductive stage compared to vegetative stage. The number of borer holes and tunnels were generally high on non-Bt plants at reproductive stage under natural infestation. This reflects greater number of egg masses recorded during reproductive stage in the second season, which coincided with the rainy season in both locations.

Recovery of Artificially Infested Larvae from Non-Bt and Bt plants

Generally, many larvae were recovered from the non-Bt plants (GA21) but none were collected from the Bt plants (Bt11xGA21) in both locations (Table 4). This again demonstrated the consistent resistant reaction of the Bt corn hybrids to the pest.

Based on the results of the eight trials in both locations, a large number of larvae were recovered during the reproductive stage of the corn plant. This is attributed to different factors, namely: (1) the readily available young and soft tassel that serve as initial food source contributes largely to their survival; (2) there were more sources of stalk available during this period as the maturing larvae prefer to bore on or into the stalks; and (3) once inside the stalk, the larvae are more protected from predation and parasitism. On the other hand**,** larval survival was lower at the vegetative stage probably due to the absence of food sources for the developing larvae (i.e., stalk boring behavior usually starts during the third instar), the higher risks of exposure to predators and other environmental factors during leaf feeding, and the greater competition for whorl feeding for the initial 50 larvae that were infested.

The above results showed that Bt plants (Bt11xGA21) are resistant to the larval feeding of ACB and confirmed the consistent efficacy of the Bt product to the corn borer. Efficacy of Bt11xGA21 was first tested under greenhouse conditions by Rasco et al. (2004). They clearly demonstrated the high efficacy of this corn product which contains the Cry1Ab protein against the ACB. Fifteen years after the Bt corn product was commercially introduced into the market, Bt11xGA21, remains effective against this pest.

Pooled Data per Plot for the Number of Larvae Recovered

Analysis of log transformed data on the number of recovered larvae showed that the main effects were significant for plant stages, infestations, and treatments. No significant difference was observed for locations and seasons (Table 5).

Overall, based on the eight trials conducted at two locations during the wet and dry seasons, significantly more larvae were recovered during the reproductive stage and a large number of larvae were recorded in the GenSan trials.

SUMMARY AND CONCLUSION

Field studies were conducted to determine the efficacy of single trait Bt corn product (Bt11xGA21) against second instar Asian corn borer at two locations, namely: Cauayan, Isabela and General Santos City, South Cotabato, two corn stages (vegetative and reproductive), and during both seasons (dry and wet). Damage ratings at five and 10 DAI were high on non-Bt plants but none on

Table 3. Mean (n=100) number of Asian corn borer holes and mean tunnel length (cm) on non-Bt and Bt corn plants at vegetative and reproductive stages under artificial and natural infestation during the first and second seasons in Cauayan, Isabela and General Santos City (GenSan), South Cotabato.*

Location	Season	Stage	Artificial Infestation		Natural Infestation	
			Non-Bt[a]	**Bt**[b]	**Non-Bt**[a]	**Bt**[b]
			Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Cauayan	First	**Vegetative**				
		No. of holes/plot	95.00 ± 13.00	0.00 ± 0.00	-	-
		Tunnel Length (cm)	0.20 ± 0.03	0.00 ± 0.00	-	-
		Reproductive				
		No. of holes/plot	371.00 ± 2.96	0.00 ± 0.00	-	-
		Tunnel Length (cm)	1.32 ± 0.06	0.00 ± 0.00	-	-
	Second	**Vegetative**				
		No. of holes/plot	12.00 ± 4.26	0.00 ± 0.00	3.00 ± 0.88	0.00 ± 0.00
		Tunnel Length (cm)	0.04 ± 0.01	0.00 ± 0.00	0.01 ± 0.00	0.00 ± 0.00
		Reproductive				
		No. of holes/plot	159.00 ± 32.27	0.00 ± 0.00	35.00 ± 14.67	0.00 ± 0.00
		Tunnel Length (cm)	0.27 ± 0.07	0.00 ± 0.00	0.09 ± 0.04	0.00 ± 0.00
GenSan	First	**Vegetative**				
		No. of holes/plot	69.00 ± 19.22	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
		Tunnel Length (cm)	33.40 ± 0.38	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
		Reproductive				
		No. of holes/plot	601.00 ± 15.88	0.00 ± 0.00	28.00 ± 5.36	0.00 ± 0.00
		Tunnel Length (cm)	49.53 ± 3.15	0.00 ± 0.00	57.00 ± 0.85	0.00 ± 0.00
	Second	**Vegetative**				
		No. of holes/plot	106.00 ± 30.95	0.00 ± 0.00	55.00 ± 3.46	0.00 ± 0.00
		Tunnel Length (cm)	26.74 ± 2.63	0.00 ± 0.00	51.70 ± 2.09	0.00 ± 0.00
		Reproductive				
		No. of holes/plot	367.00 ± 12.57	0.00 ± 0.00	463.00 ± 7.84	0.00 ± 0.00
		Tunnel Length (cm)	56.78 ± 0.72	0.00 ± 0.00	58.25 ± 0.84	0.00 ± 0.00

*Means within each trait followed by a common letter are not significantly different at 5% level by Tukey's test.

Table 4. Mean number of Asian corn borer larvae recovered on Non-Bt and Bt corn plants at vegetative and reproductive stages under artificial and natural infestation during the first and second seasons in Cauayan, Isabela and General Santos City (GenSan), South Cotabato.*

Location	Season	Stage	Artificial Infestation		Natural Infestation	
			Non-Bt[a]	Bt[b]	Non-Bt[a]	Bt[b]
			Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Cauayan	First	Vegetative	127.33 ± 9.67	0.00 ± 0.00	-	-
		Reproductive	799.33 ± 55.90	0.00 ± 0.00	-	-
	Second	Vegetative	22.67 ± 9.17	0.00 ± 0.00	35.00 ± 35.68	0.00 ± 0.00
		Reproductive	141.67 ± 62.40	0.00 ± 0.00	32.00 ± 22.61	0.00 ± 0.00
GenSan	First	Vegetative	179.33 ± 82.20	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
		Reproductive	1535.00 ± 227.49	0.00 ± 0.00	83.67 ± 17.04	0.00 ± 0.00
	Second	Vegetative	534.00 ± 201.55	0.00 ± 0.00	101.67 ± 17.47	0.00 ± 0.00
		Reproductive	643.33 ± 86.93	0.00 ± 0.00	1099.33 ± 87.37	0.00 ± 0.00

*Means within each trait followed by a common letter are not significantly different at 5% level by Tukey's test.

Table 5. Analysis of variance of Log transformed recovered larvae.

Source of Variation	Degrees of Freedom	Seq Sum of Squares	Adj Sum of Squares	Adj Mean Square	F	P
Location	1	0.67	0.32	0.32	2.05	0.160
Replication	4	0.30	030	030	0.47	0.750
Season	1	0.06	0.00	0.00	0.00	0.950
Stage	1	3.37	3.03	3.03	19.43	0.000**
Infestation	1	4.19	1.69	1.69	10.88	0.002**
Treatment	1	90.90	73.18	73.18	470.06	0.000**

** Means main effect is significant at 0.01 level.

Bt plants at both vegetative and reproductive stages for the first and second trials in both sites. Generally, many borer holes and tunnels were recorded on non-Bt plants but not on Bt plants, which did not show any sign of boring and tunneling. Therefore, Bt corn plants are resistant to feeding of ACB larvae, and the results confirmed the consistent efficacy of this product against this corn pest.

Damage ratings showed that there were significant main effects between plant stages, infestations, and treatments. There were no significant differences between locations (Cauayan vs. GenSan) and seasons (wet vs. dry). Data on recovered larvae showed that the main effects were similarly significant for plant stages, infestations and treatments. Also, no significant differences were observed between locations and seasons. Significantly, no larvae were recovered from Bt plants while many were recovered from non-Bt plants under both the naturally and artificially infested plots. Naturally infested plots had significantly fewer recovered larvae than the artificially infested plots.

The results of all the eight field bioefficacy trials at two locations showed that Bt plants (Bt11xGA21) are highly resistant and the non-Bt plants (GA21) are susceptible to the second instar larvae. The single Bt trait corn product, Bt11xGA21, remains effective against the Asian corn borer. The results from these bioefficacy trials clearly demonstrate the consistent performance of Bt11xGA21 in controlling this corn pest.

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