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RESPONSE OF SECOND INSTAR ASIAN CORN BORER, *Ostrinia furnacalis* (Guenee) (CRAMBIDAE: LEPIDOPTERA), TO Bt TRANSGENIC MAIZE, MON89034

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ABSTRACT

Response of the Asian corn borer (ACB) to the Bt corn hybrid, MON89034, was determined using leaf disc and whole plant bio-assays at the vegetative stage against second instars of Isabela and Laguna ACB populations. Survival of ACB larvae was low on Bt corn while survival was consistently higher on non-Bt corn, NK603. No significant difference was observed between Laguna and Isabela ACB populations. Results showed that the transgenic corn hybrid, MON89034, may provide protection against the second instars of the ACB.

Key words: Asian corn borer populations, Bt corn, leaf disc bioassay, *Ostrinia furnacalis*, whole plant bioassay

INTRODUCTION

Bt corn hybrids have improved production through enhanced host resistance to lepidopterous pests. One of the pests managed by these corn hybrids is the Asian corn borer (ACB) *Ostrinia furnacalis* (Guenee), the most important insect pest of corn (Mendelsohn et al., 2003). The ACB can cause losses in corn production from 44 to 100% (Yorobe & Quicoy, 2006; Caasi-Lit et al., 1987). The soil bacterium, *Bacillus thuringiensis* Berliner (Bt), has long been utilized as a microbial insecticide to control lepidopteran pests of corn. However, spray methods using Bt formulations are effective only when insects feed at the surface of the corn plants. Corn borer larvae, from the second to fifth instars, feed inside the stalk of corn rendering Bt formulations less effective. The introduction of the bacterium's genes controlling the expression of the endotoxin protein to the corn plant genome offered a systemic, built-in control against the pest.

MON89034 is a second generation insect resistant corn developed to provide protection against lepidopteran pests (Monsanto, 2009). This stacked transgenic corn hybrid expressing Bt Cry1A.105 and Cry2Ab2 proteins, has consistently provided excellent protection against the European corn borer (Rule et al., 2014). Based on several laboratory and field studies, Bt corn MON89034 also provided effective control against ACB in the Philippines (Caasi-Lit, 2014). The effects were initially confirmed in previous efficacy data in the greenhouse by Solsoloy et al. (2007). Furthermore, field efficacy data showed that MON89034 was effective against ACB during the wet (2008) and dry (2009) cropping seasons in different corn production areas in the country (Samson et al., 2009). Several years after it was approved for field commercialization, the same results indicated that MON89034 was still effective in controlling the ACB in all corn growing regions.

The objective of this paper is to determine the efficacy of the Bt corn hybrid, MON89034, compared to a non-Bt control, NK603, using leaf disc and whole plant bioassays at vegetative stage of the corn plant against Isabela and Laguna ACB populations.

MATERIALS AND METHODS

Establishment of initial ACB populations

Initial populations of ACB were collected from different locations in Laguna and Isabela provinces. Collected specimens were an assortment of different insect stages ranging from egg masses to pupae. Laguna populations were collected from three different sites at the Institute of Plant Breeding (IPB) Experimental Station in Tranca, Bay, Laguna, from January 13 to February 24, 2015 (Table 1). Most of the collections were pupae and late instar larvae found in corn stalks. Collections from Isabela were late instar larvae found in corn stalks and egg masses on the leaves.

Table 1. Initial collections of Asian corn borer (ACB) from corn plants for the establishment of laboratory rearing of test insects for the efficacy study of MON89034 against second instar ACB larvae.

Place of Collection	Collection Date	Collected ACB stage	Plant Parts
Laguna Province			
Tranca, Bay	January 13, 2015	19 pupae	stalks
		86 larvae	stalks
	January 14, 2015	24 pupae	stalks
		54 larvae	stalks
	February 24, 2015	3 egg masses	leaves
		11 pupae	stalks
		6 larvae	stalks
Isabela Province			
Cauayan	January 9, 2015	103 larvae	stalks
	February 2, 2015	34 egg masses	leaves

In order to establish laboratory colonies, field collected larvae, pupae, and egg masses were sorted and placed in separate containers. Sexes of pupae were determined before placing them into ovipositional cages. Adults were fed sugar solution from cotton balls. Larvae were placed in pans with IPB modified artificial diet (Caasi-Lit et al., 2015). Egg masses were placed in Petri dishes lined with moistened filter paper. Upon hatching, larvae were fed with artificial diet. Rearing procedures followed that of Caasi-Lit et al. (2015). For biosafety purposes, the Laguna and Isabela populations were assigned to separate secured rearing rooms inside the IPB Entomology Laboratory.

Leaf Disc Bioassay

Test insects were laboratory-reared, second generation, second instar ACB larvae. They were initially fed artificial diets and transferred onto leaf discs after four days.

The test plant materials were leaf discs from greenhouse-grown corn plants, 30 days after planting. The young, pale green to yellow colored leaves, usually found on the whorl were cut from the middle portion of the plant. These were cut into circular discs using a small metal cylindrical leaf disc cutter with a diameter of 2.3 cm. The edge of the cylinder cutter was pressed onto the leaf and was rotated until the leaf disc was detached from the whole leaf. Leaf discs were placed into Petri dishes with moistened filter paper at the bottom to keep the leaves fresh. Fifty leaf discs were prepared for each Bt and non-Bt treatment. One trial each for Laguna and Isabela ACB populations was conducted. Trial procedures were adapted from the improved leaf disc assay technique developed in Phase I of this project (Caasi-Lit, 2014).

One neonate was transferred from the source container onto each of the 50 leaf disc replicates using a fine camel-hair brush. The Petri dishes were covered and labeled with VAR1 (for Bt corn) and VAR2 (for non-Bt corn) including the replicate numbers. Petri dishes (8.3 cm in diameter, 1.2 cm deep) were wrapped with clear plastic film (GLAD® Cling Wrap) before covering to prevent the larvae from escaping. Petri dishes were kept at ambient room conditions (about 28.9˚C, 68% RH) with 12-hour daylight. The Petri dishes were observed and photographed, and the number of dead and live larvae counted daily for seven days.

Larval survival was computed as the number of larvae that survived over the total number of larvae infested multiplied by 100.

Whole Plant Bioassay

Second generation, second instar ACB were reared on an artificial diet (Caasi-Lit et al., 2015) in the laboratory and used for the bioassay. Corn plants at 30 days after planting were used as test plants. Thirty second-instar ACB larvae were placed onto the whorls of 90 Bt and non-Bt corn test plants.

The plants were observed and the damage per leaf was evaluated for five days. On the first, third, and fifth days, 30 plants were examined to determine the survival of the infested larvae on both Bt and non-Bt plants. The rating scale (% damage on the leaf) used is shown in Table 2. Ninety replicates each were carried out for Bt and non-Bt plants.

Table 2. Damage descriptions and ratings for the whole plant bioassay (Caasi-Lit et al., 2014).

Quantitative Description	Qualitative Description	Damage Rating
≥ 31%	Presence of long lesions already showing advanced scrapings leaving fine films on abaxial epidermis	5
21-30 %	Mostly shot-holes and match-holes and some shorter lesions start to appear	4
11-20 %	Mostly pin-holes on leaves	3
3-10 %	Mostly scores, scrapes, nibbles, dots observed on leaves	2
1- 2 %	Only few tiny faint scores, scratch or scrapes on outermost layer of adaxial epidermis	1
0	No damage	0

Damage was evaluated following the rating scale from Caasi-Lit et al. (2014). Damage ratings were evaluated for each plant leaf stage (the inner whorl, fully expanded leaves [EL] and all other remaining leaves). Overall damage was computed by averaging the results for each plant.

Statistical Analysis

Using MS Excel 2007, data on percent larval survival were analyzed using one-way ANOVA. Treatment differences were determined using t-test at α=0.05. Pooled data on larval survival was analyzed using SPSS ver. 19.

RESULTS AND DISCUSSION

Leaf Disc Assay

Figures 1 and 2 show the daily mean survival of ACB larvae, respective progenies of initial collections from Laguna and Isabela, and fed with leaf disc from the whorls of Bt and non-Bt plants for seven days.

For the Laguna population, the survival of larvae on Bt were 100% and 98% on the first and second day after infestation, respectively, while mean larval survival of ACB was 0% on the third up to the seventh day (Figure 1). On the other hand, the survival of the ACB larvae infested on NK603 was all 100% from the onset of infestation until the end of the 7-day observation period. This indicates a significant decline in the number of larvae accompanied by almost no damage, rather only light and tiny pinches observed on Bt corn even just after the third day.

For the Isabela population, the mean survival of ACB larvae fed with Bt and non-Bt were 66% on the first day, 4% on the second day and 0% from the third day onwards (Figure 2). All larvae survived on non-Bt corn. Comparing the survival of ACB between populations, more larvae survived using Laguna population (mean of 99%) as compared to the reduced survival during the first two days of bioassay using the Isabela population.

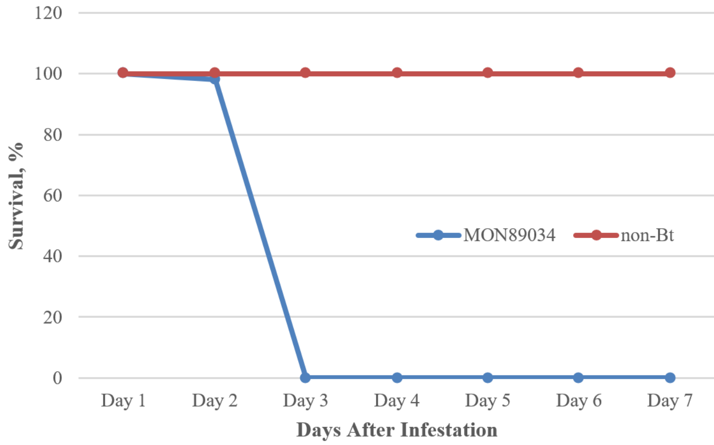

Figure 1. Mean larval survival (%) of second instar Laguna ACB fed with leaf discs of Bt corn (MON89034) and non-Bt corn (NK603) for the 7-day experiment.

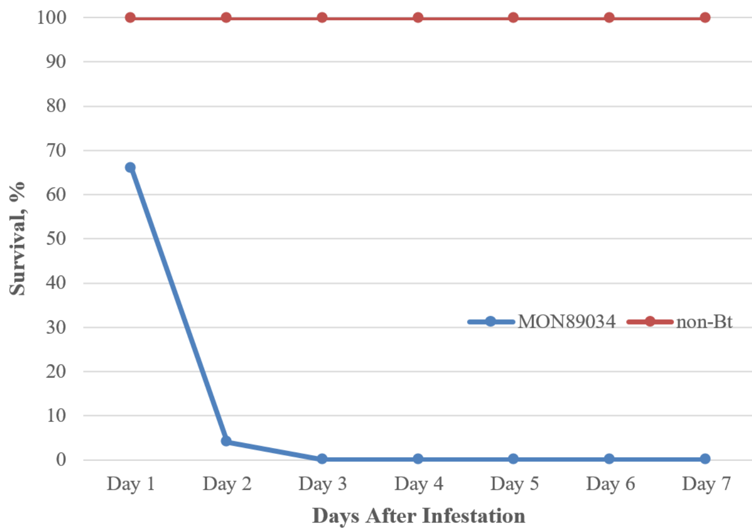

Figure 2. Mean larval survival (%) of second instar Isabela ACB fed with leaf discs of Bt corn (MON89034) and non-Bt corn (NK603) for the 7-day experiment.

The results for the two populations show the same significant trend where leaf discs of Bt corn were least damaged while those of non-Bt were all consumed by second instar larvae. Larval survival rates were very low and declining on Bt corn (MON89034) but consistently high on non-Bt corn (NK603) from the onset of infestation. This observation is also consistent with the results in the previous experiments (Caasi-Lit, 2014). The significant results obtained suggest that this Bt transgenic corn hybrid remains effective in controlling ACB.

Whole Plant Assay

Using whole plant assay on the Laguna population, damage by ACB larvae differed significantly among the leaf stages of Bt and non-Bt corn, with the inner whorl having the most damage followed by the fully expanded leaves. Damage on Bt transgenic corn was lower than that on non-Bt corn (Table 3). Mean damage five days after infestation on non-Bt corn was 4.30, 3.12, and zero for the whorl, fully expanded leaves, and all the other remaining leaves, respectively. Damage for all the leaf stages on Bt corn were all less than damage level 1. The damage is characterized by the presence of small scrapes and very few dots and tiny holes. Dead (shrinking) larvae were found on the leaves two days after infestation. On the other hand, the damage on non-Bt corn was progressing; pin-holes/shot-holes and lesions were observed on the leaves. Mean damage ratings on non-Bt corn were significantly higher on all leaf types (Figure 3).

For the Isabela population, damage on Bt and non-Bt corn differed significantly among the different leaf stages (Table 4). Damage rating on Bt corn was significantly lower compared to that on non-Bt corn. For all leaf stages, mean damage ratings were significantly higher on non-Bt corn (Figure 4).

Results of the whole plant assay showed that MON89034 had low damage rating compared to the higher damage ratings on NK603. The second ACB instar larvae from Isabela population survived up to seven days on non-Bt corn but only for two days on Bt corn. There was very slight damage or none at all on Bt corn as shown by the tiny scrapes while leaf damage remarkably progressed on non-Bt corn for the 7-day assay period. Similar results were obtained using the Laguna population.

Pooled data for whole plant assay

Pooled data revealed that there was no significant difference between the Laguna and Isabela ACB populations (Table 5).

For the Isabela population, the second instar larvae survived up to five days on non-Bt corn and only for two days on Bt corn. The progression of damage on Bt corn was for three days only, and stopped, while that on non-Bt corn was evident until the fifth day. Nevertheless, significantly more larvae generally survived using the Laguna than the Isabela population (Figure 3). For the Laguna population, where Bt pressure is less in the originating crop environment, higher larval survival and leaf feeding damage were observed compared to the Isabela population (Figure 4).

Table 3. Damage on several leaf stages of Bt and non-Bt corn hybrids for 5-day sampling period by Laguna Asian Corn Borer population.

Var/Leaf Stage	Damage				
	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**
Non-Bt (whorl)	1.55a	2.31a	2.62a	3.25a	4.3a
Bt (whorl)	0.77b	0.92b	0.92b	0.88b	0.88b
P value	4.13E-06	4.14E-11	8.37E-13	0.0025	2.51E-06
Non-Bt (Fully EL)	0.92a	1.27a	1.52a	2.0a	3.12a
Bt (Fully EL)	0.16a	0.31a	0.31?	0.23b	0.23b
P value	0.0007	6.65E-05	0.0010	4.34E-05	2.09E-05
Non-Bt (others)	0.23a	0.7a	0.94a	1a	1.24a
Bt (others)	0.00a	0.02a	0.00	0.00a	0.00a
P value	<0.001	<0.001	8.91E-12	<0.001	<0.001

EL – expanded leaves

Table 4. Damage on several leaf stages of Bt and non-Bt corn hybrids for 5-day sampling period by Isabela ACB population.

Var/Leaf Stage	Damage				
	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**
Non-Bt (whorl)	1.21a	1.85a	2.55a	2.88a	4.4a
Bt (whorl)	0.98a	0.98b	0.98b	0.97b	0.97b
P value	<0.001	<0.001	<0.001	8.14^{E-11}	1.74^{E-12}
Non-Bt (Fully EL)	1.02a	1.29a	1.48a	1.82a	2.53a
Bt (Fully EL)	0.17a	0.16b	0.16b	0.18b	0.19b
P value	0.008542	<0.001	0.009	6.70^{E-05}	7.23^{E-05}
Non-Bt (others)	0.17	0.54	1.64	0.98	1.04
Bt (others)	0.00	0.00	0.00	0.00	0.00
P value	<0.001	<0.001	<0.001	<0.001	<0.001

EL – expanded leaves

Table 5. ANOVA for the whole plant assay.

Source	Type III Sum of Squares	df	Mean Square	F	Significance
Intercept	55.013	1	55.013	84.683	0.000
Site	.036	1	.036	.055	0.822 n.s.
Treatment	21.165	1	21.165	32.581	0.001 **
Leaf stage	20.456	2	10.228	15.744	0.003 **
Error	4.547	7	.650		

n.s.-not significant; **- significant at 0.05 level of significance.

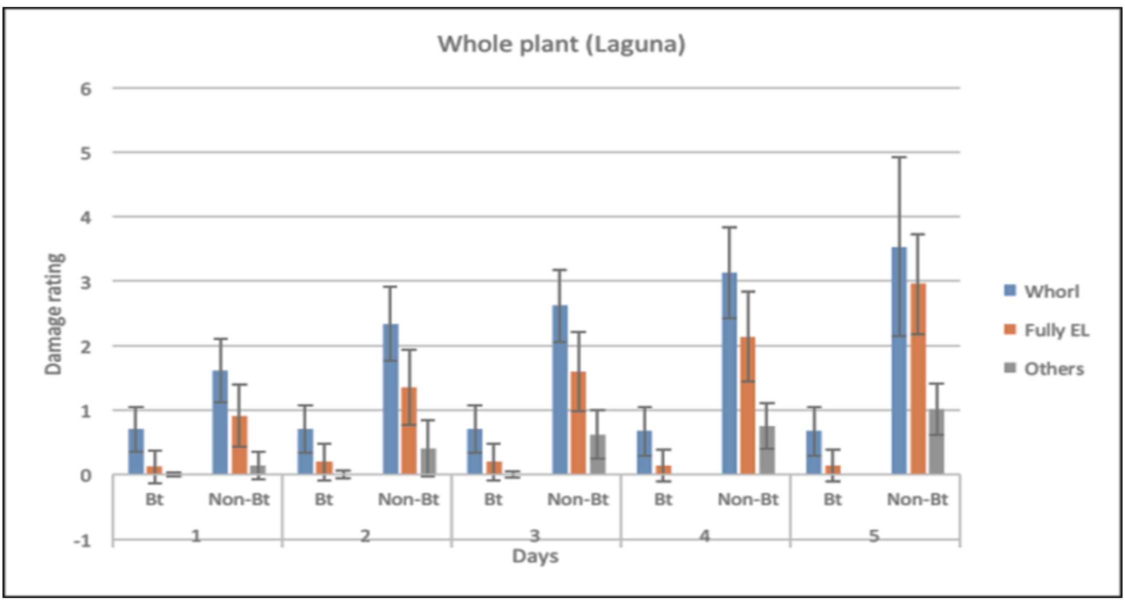

Figure 3. Mean leaf feeding damage of ACB Laguna larvae on several leaf stages of Bt and non-Bt whole plants for 5 days.

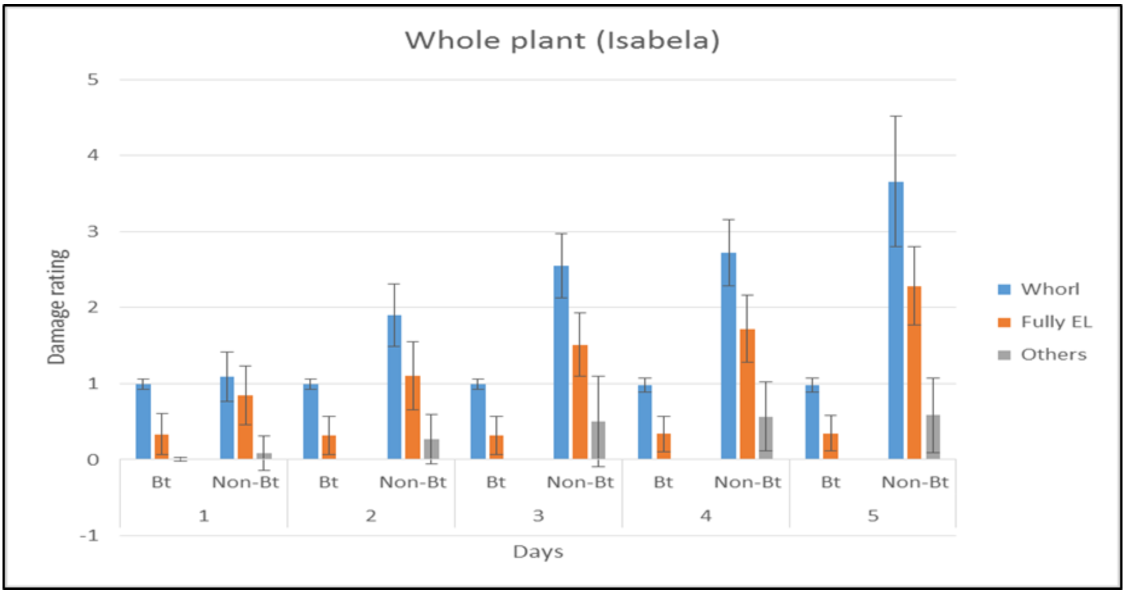

Figure 4. Mean leaf feeding damage of ACB Isabela larvae on several leaf stages of Bt and non-Bt whole plants for 5 days.

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