AN IMPROVED TECHNIQUE FOR ARTIFICIAL INFESTATION OF TEST INSECTS IN BIOEFFICACY STUDIES OF Bt CORN AGAINST THE ASIAN CORN BORER, *Ostrinia furnacalis* (Guenee), IN THE PHILIPPINES

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ABSTRACT

Pest-resistant crops are identified using bioefficacy studies, that entail the infestation of test insects onto candidate plants to screen for the desired resistance trait. A technique using cornstalks on biodegradable skewers was developed to test the efficacy of Bt transgenic corn against the Asian corn borer, *Ostrinia furnacalis* (Guenee) (Lepidoptera: Crambidae). Stalks of isoline maize, aged 30-40 days after planting, were sliced to pieces, each about 1 cm thick, and skewered into pre-thinned barbecue sticks. The skewered corn stalks were placed into the infestation cup, and the newly-hatched larvae were transferred using camel-hair brush. The cups were covered with tissue paper then stored for several days. During infestation, the skewered cornstalks were placed inside the whorl for vegetative stage infestation, or on the leaf sheath just below the flag leaf for reproductive stage infestation. Four field trials were conducted to test the effectiveness and efficiency of this technique. Consistently low variation in the damage ratings were observed from sets of plants under the same treatment. The standard deviations of damage ratings obtained were relatively low regardless of the site of conduct, the trial, the stage of corn, and the day of observation. This infestation technique is a better delivery system that improves handling of test insects and, therefore, allows greater larval survival, and decreases variation in the data collected.

Key words: Asian corn borer, bioefficacy, cornstalks, infestation technique, *Ostrinia furnacalis*

INTRODUCTION

The search for pest-resistant crops requires the screening and bioefficacy testing of candidate crops. Bt transgenic corn hybrids should be tested for their efficacy against target insects under local conditions before they are released commercially. In the Philippines, the

Asian corn borer (ACB), *Ostrinia furnacalis* (Guenee), is the most destructive insect pest of corn (Caasi-Lit et al., 1989) and tests are needed to evaluate and understand the resistance of the crops to the ACB.

In previous years, artificial infestation of corn borers was done by placing, or sometimes pinning, the egg masses into the whorl of the plant (Guthrie et al., 1982), or by manually placing the larvae into the plant using a camel-hair brush (Wiseman et al., 1966). These techniques, however, are inefficient and labor-intensive. Thus, it was difficult to conduct large-scale screening for insect resistance (Wiseman et al., 1974). In this light, the bazooka technique was developed by Mihm (1982) as an efficient method for infestation, originally for the corn earworm. Mihm's (1982) technique involves the mixing of larvae with corn grits and then dispensing them into the corn plant using a special dispenser (Caasi-Lit & Lontoc, 2014, unpublished data), consequently removing laborious steps such as cutting, pinning, and manipulation of larvae using camel-hair brush (Mihm, 1987). Bazooka technique is heavily dependent on the quality of the medium used (e.g., corn grits), and the precise number of larvae introduced on each plant. Varying the number may affect the uniformity of the damage from each sample which may compromise the quality of data that would be generated (Caasi-Lit & Lontoc, 2014, unpublished data). At present, studies on delivery systems for test insects are limited in the Philippines. Techniques used abroad and/or for other lepidopterous pests of corn (e.g. Davis, 1980; Guthrie et al., 1960; Mihm, 1983; Ortega et al., 1980; Widstrom, 1967) are adopted without proper local evaluation.

To test the effectiveness of different infestation techniques commonly used in the Philippines, Caasi-Lit & Lontoc (2014, unpublished data) compared the use of camel-hair brush, bazooka, cornstalks and egg masses (blackhead stage) as delivery systems for neonate ACB larvae for bioefficacy testing. They found that larvae in cornstalks applied to corn at 25-30 days after planting (DAP) and egg masses applied to 50-DAP corn had the highest larval recovery. However, both delivery systems entail laborious preparation while the use of bazooka gave the most erratic results due to the quality of corn grits used.

There is clearly the need for a more efficient delivery system that reduces unwanted mortality of test insects, is less laborious both in terms of preparing materials and in the field during the delivery proper, and helps minimize sources of variation in experiments. In addition, under Philippine conditions, the materials needed should be economical/affordable and readily available.

This paper, therefore, aimed to: (a) describe the technique we have developed and hereby nickname "cornstalk barbecue" and the procedures in preparing the needed materials; and (b) present data from several field trials to confirm the effectiveness and efficiency of the technique.

MATERIALS AND METHODS

Materials used and their sources

All plant materials were obtained from Syngenta Philippines Inc., and grown and maintained using standard agronomic practices. The test insects used were from Isabela and South Cotabato ACB populations reared using improved artificial medium (Caasi-Lit et al., 2015) in the Entomology Laboratory of the Institute of Plant Breeding, College of Agriculture and Food Science, University of the Philippines Los Baños (UPLB), College, Laguna.

Field trials

Two trials were conducted in two locations – Cauayan City in Isabela Province (hereinafter referred to as "Isabela") and General Santos City in South Cotabato Province (hereinafter referred to as "GenSan"). The plots for the trials were laid out in three replications. Each plot had seven rows, each 4.60 m long, with the two outermost rows (1 and 7) serving as borders. Data were gathered from the five inner rows. Plant spacing was 0.70 m between rows and 0.20 m between hills. Since there were 24 plants per row, a total of 168 plants per plot were infested with second instar ACB larvae at both vegetative (25-30 DAP) and reproductive (40-45 DAP) stages. The infestation rate was 50 and 30 larvae per plant at vegetative and reproductive stages, respectively. Plots were then enclosed with screen nets measuring 6 x 6 x 2.5 m for protection from natural enemies and from further infestation by ACB.

Egg masses preparation

Laboratory-reared egg masses were collected from the oviposition cages, cut from the wax paper and arranged in Petri dishes lined with moist filter paper (Figure 1A). The Petri dishes were kept inside the rearing pans for 3-4 days to incubate the egg masses (Figure 1B). By the end of the incubation period, egg masses turned into blackheads (Figure 1C) and later hatched as neonate larvae (Figure 1D).

Cornstalk barbecue preparation

Cornstalks were obtained from 30-40 day old non-Bt corn plants. The leaves were removed and the stalks were cut into slices, each about 1 cm thick (Figure 2A). Regular sized toothpicks or broom sticks were divided and used to skewer the sliced stalks (Figure 2B). The resulting cornstalk barbecues (Figure 2C) were placed in a cup together with young leaf tissues (Figure 2D). A piece of tissue paper was placed on top of the cup prior to capping with the lid (Figure 2E).

Infestation of cornstalk barbecues

Blackhead egg masses were taken out from the rearing pans. Shortly, larvae started to come-out from their egg shells. Using a camel-hair brush, around 20-30 neonates were individually transferred to each cup containing a single cornstalk barbeque. The cups were covered with tissue paper, fastened with a lid, and stacked in plastic containers. The plastic containers were stored in the laboratory for several days to allow the larvae to settle and develop to the desired larval instar for infestation. Cornstalk barbecues were replaced every two days after seeding until ready for infestation.

Field infestation using cornstalk barbecue

For infestation of vegetative-stage corn, the cornstalk barbecues were placed inside the whorl (Figure 3A and 3B). However, during the rainy season, the barbecues were pinned on the upper portion of the whorl to avoid soaking in water. For infestation on reproductive-stage corn, the cornstalk barbecues were placed on the leaf sheaths just below the flag leaf (Figure 3C). Once the stalk dried up, the larvae were forced to transfer into the whorl or inside the leaf sheaths and thus directly feed on the leaves of the non-Bt (=isoline or susceptible) entry (Figure 3D).

Figure 1. Preparation of laboratory-reared egg masses: (A) arranging egg masses on Petri dishes; (B) rearing pans; (C) Asian Corn Borer (ACB) egg mass at the blackhead (pre-hatching) stage; (D) newly-hatched ACB larvae.

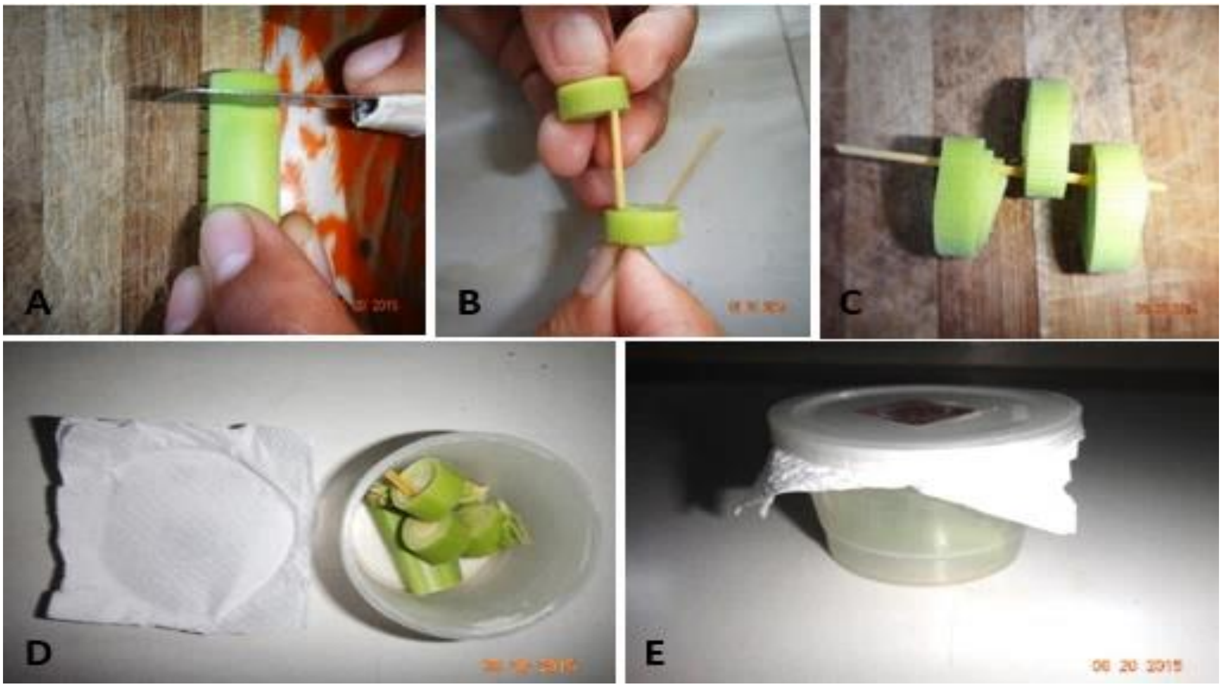

Figure 2. Preparation of cornstalk barbecue: (A) cutting a corn stalk in to 1 cm thick slices; (B) skewering the corn stalk slices using barbecue sticks; (C) a prepared cornstalk barbecue; (D) cornstalk barbecue inside the cup; (E) sealed cup with cornstalk barbecue.

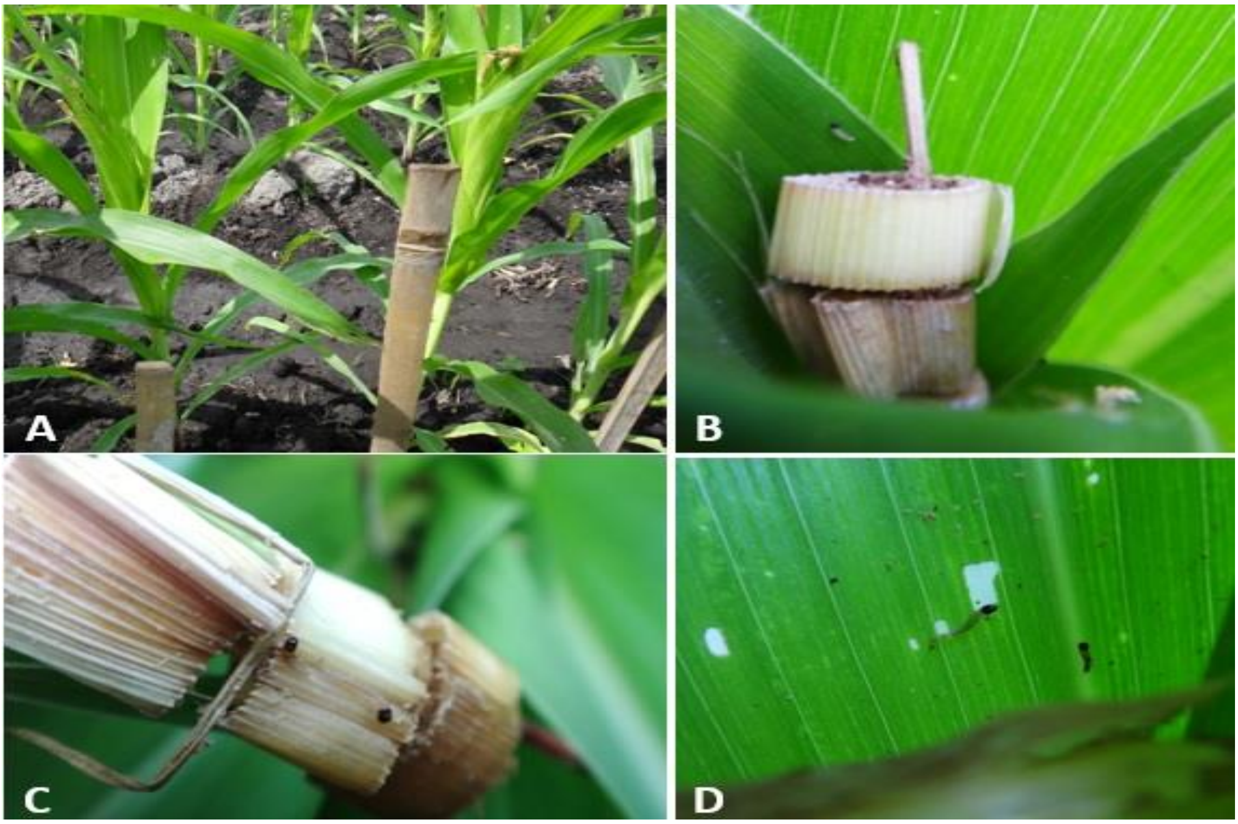

Figure 3. Field infestation of Asian Corn Borer larvae using cornstalk barbecues: (A) during the vegetative stage; (B) cornstalk barbecue inside the whorl; (C) cornstalk barbecue on the leaf sheaths at the reproductive stage; (D) surviving larvae transferring to and feeding on the leaves.

Evaluation of the infestation technique

After infestation, damage caused by the infested larvae on each plant was assessed at 5 and 10 days after infestation (DAI). Damages on the leaves at 5 and 10 DAI for the vegetative stage, and at 5 DAI for the reproductive stage were rated using the leaf-feeding damage rating scale presented in Table 1. On the other hand, damage at 10 DAI for the reproductive stage was rated using the stalk-feeding damage rating scale shown in Table 2. The obtained ratings were assessed for preciseness using standard deviation from the mean damage ratings as the statistical method.

Table 1. Leaf feeding damage rating scale (Wiseman et al., 1966) used at the 5 and 10 DAI damage rating periods of plants infested at vegetative stage, and at the 5 DAI damage rating of plants infested at reproductive stage.

Resistance level	**Rating**	**Description**
Highly Resistant	0	No damage
	1	Few pinholes
Resistant	2	Several to many pinholes
	3	Few shot holes & 1 or 2 elongated lesions
Intermediate	4	Several shot holes & few elongated lesions
	5	Several shot holes & elongated lesions
Susceptible	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 very visible drying
Highly Susceptible	9	Whorl almost eaten, plenty of elongated lesions, leaves dry & dying
	10	Dying or dead plants

Table 2. Stalk-feeding damage rating scale used at the 10 DAI damage rating of plants infested at reproductive stage.

Resistance level	**Rating**	**Description**
Highly resistant	0	No damage
Resistant	<1	1 or 2 holes above and under the corn ear
Intermediate	1	2 holes above and under the corn ear and broken tassel; 2 to 3 holes above and under the corn ear
Susceptible	2-4	More than 3 hole above and under the corn ear and tassel broken
Highly susceptible	5-9	All nodes have holes and tassel broken

RESULTS AND DISCUSSION

Description of the technique

The cornstalk barbecue technique was developed by the Entomology Laboratory, Institute of Plant Breeding, UPLB, as an answer to the need for an alternative delivery system for second instar ACB larvae infestation. The technique uses 30-40 DAP cornstalk as it was found to be the most preferred food source of second instar larvae. For ease of delivery, the stalks, taken from the middle to the upper portion of a corn plant, were cut into small pieces (approximately 1 cm long) and then skewered into pre-thinned barbecue sticks. Durable toothpicks or pieces of sterilized coconut palm midribs from a clean Filipino stick broom ("*walis tingting*") may be used also. Each cornstalk barbecue can be infested with at most 30 larvae. The larvae are allowed to settle on the cornstalks until they reach the second-instar (4-day old). The pre-infested cornstalks are then pinned to the plant to facilitate larval transfer.

This technique is currently being used at the Entomology Laboratory for greenhouse and field studies involving bioefficacy testing of Bt and non-Bt corn against the ACB and other lepidopteran pests of corn. This is also the procedure used in the greenhouse confirmatory testing of selected potential resistant varieties for crop improvement work.

Field trial results

Based on a rating scale of 1-10, where 1 is resistant and 10 is susceptible, over all mean damage ratings between Bt and non-Bt plants in all the trials were significantly different (Caasi-Lit et al., data part of paper to be published separately). Non-Bt plants had the highest mean damage rating while Bt plants had the lowest. The second instar larvae survived on non-Bt plants up to 15 days with consistently increasing damage signs and symptoms inflicted on the plants. Only very slight pinhole damage was observed on Bt corn hybrids, and then larvae began to shrink and die after 1-2 days.

For the purpose of this paper, since all the Bt plants had a rating of 1, only the data for the non-Bt corn were analyzed and shown.

Preciseness of data observed in one sampling period

The most significant accomplishment of this study is the greatly improved precision of damage ratings obtained from two trials in two locations. For each replication, a total of 100 plants were observed. The precision or refinement of measurement of the damage ratings obtained from every individual plant for each sampling period is a good indicator of the uniform data set. The results clearly show the unprecedented degree of precision for the data generated using the cornstalk barbecue infestation technique. This means that the damage caused by the delivered test larvae on the test plants was more or less uniform such that when damage was assessed using the adopted damage rating scale, the scores or ratings were almost the same across the 100 plants. Figure 4 shows representative data from the vegetative and reproductive stages of corn in one sampling period for each stage of the corn crop.

Based on the results, test corn plants at both vegetative and reproductive stages showed relatively low standard deviations with values of 0.7818 and 0.7785, respectively (Table 3). Variance, another measure of dispersion, also showed low values for both plant stages. Low standard deviation and variance indicate higher precision of the damage ratings obtained. Hence, it can be inferred that the cornstalk barbecue infestation technique successfully delivered a consistent number of second-instar ACB larvae to the target plant thereby causing relatively even or more or less uniform damage on the test plants.

A

Plants #							
Border	0	5	0	4	5	5	0
Border	0	4	4	4	4	5	4
20	4	3	5	4	6	6	4
19	4	3	5	4	4	6	5
18	3	4	5	4	4	6	5
17	3	5	5	4	5	5	5
16	4	5	5	6	6	5	6
15	3	3	4	5	5	6	6
14	3	4	4	4	3	5	5
13	4	4	3	4	5	4	5
12	3	5	4	5	4	4	4
11	3	4	4	5	4	5	5
10	5	4	4	4	5	5	5
9	3	4	4	5	4	6	4
8	4	4	4	6	5	5	4
7	3	4	5	4	5	5	5
6	3	5	4	5	5	5	5
5	4	3	4	4	5	5	5
4	3	5	4	5	5	5	4
3	4	4	5	5	5	4	5
2	3	3	5	4	4	5	4
1	2	4	6	6	5	4	4
Border	0	4	6	4	6	5	4
Border	0	3	6	4	6	6	5

B

Plants #							
Border	0	4	3	4	4	3	0
Border	3	4	3	4	4	3	0
20	4	4	4	5	5	4	0
19	4	5	4	4	5	4	4
18	4	5	3	3	3	5	3
17	3	5	4	5	5	4	3
16	3	3	4	4	4	3	3
15	3	5	4	3	4	5	4
14	3	4	3	5	5	4	3
13	4	4	5	4	5	3	3
12	5	5	4	4	4	3	4
11	4	5	4	4	4	3	4
10	4	3	5	4	5	4	5
9	3	3	4	3	5	4	5
8	3	4	3	3	5	5	3
7	4	4	5	3	4	5	5
6	4	3	4	5	4	5	4
5	4	3	4	3	3	4	4
4	3	5	4	3	3	3	5
3	3	5	4	3	3	4	3
2	4	4	3	4	4	5	3
1	3	3	3	3	3	5	3
Border	3	5	3	4	4	4	3
Border	3	4	3	5	3	3	0

Figure 4. Representative data on damage ratings generated from the (A) vegetative and (B) reproductive stages of corn in one sampling period; highlighted in orange are the test plants.

Table 3. Statistical summary of representative data on damage ratings generated from one sampling period.

Parameter	Vegetative stage of corn	Reproductive stage of corn
Mean damage ratings	4.57	4
Median	5	4
Measures of dispersion		
Range	3-6	3-5
Standard deviation	0.781800564	0.778498944
Variance	0.611212121	0.606060606
Coefficient of variation	17.10%	19.46%
Skewedness	-1.65	0
Standard error of the mean	0.07818	0.07785

Figure 5 shows normal frequency distribution among the damage ratings for the vegetative and reproductive corn plant, with medians at 5 and 4, respectively. The means were also calculated for both plant stages, and had values of 4.57 and 4 for the vegetative and reproductive stages, respectively. Computed standard errors of the mean for both plant stages indicate high precision of the mean value.

Asian corn borer damage

Figures 6 and 7 show the nature of damage on the test plants by the delivered/infested larvae at the vegetative and reproductive stages, respectively. The progression of damage from 5 DAI to the next rating period (10 DAI) demonstrates the details enumerated in the damage rating scales presented in Tables 1 and 2. Damage by ACB feeding on the leaves is characterized by pinholes, shot holes, and lesions. The feeding sites were distinctively mainly inside the whorl where the cornstalk barbecue sticks bearing the test larvae were placed during the artificial infestation (Figures 6A and 6B). Once the leaves became fully expanded, the damage became more pronounced and visible. At the reproductive stage, the damage is mostly characterized by the presence of entry holes near the internodes, the feeding site of the larvae that start to bore into the stalk (Figures 7A and 7B).

Summary data for the two trials in two locations

Two damage rating scales were used in the study to account for leaf-feeding and stalk-feeding damage. For the entire damage rating during the vegetative stage, the leaf-feeding scale was used. However, for the reproductive stage, the leaf-feeding scale was used for the 5 DAI rating and the stalk-feeding scale for the 10 DAI rating – when stalk-feeding is more significant than leaf-feeding.

Table 4 shows the summary of the damage ratings taken at the vegetative stage of corn in two trials in two locations. Regardless of the trial and experimental site, the generated damage ratings showed consistent substantial progression of damage from 5 DAI to 10 DAI (Figure 6; Table 4). Moreover, the standard deviations of damage ratings generated from this plant growth stage in both trials, locations, and day of observation, ranges from 0.09 to 0.42. Considering that the range of the damage rating used is from 0 to 10, this relatively low standard deviation suggests preciseness of the gathered data.

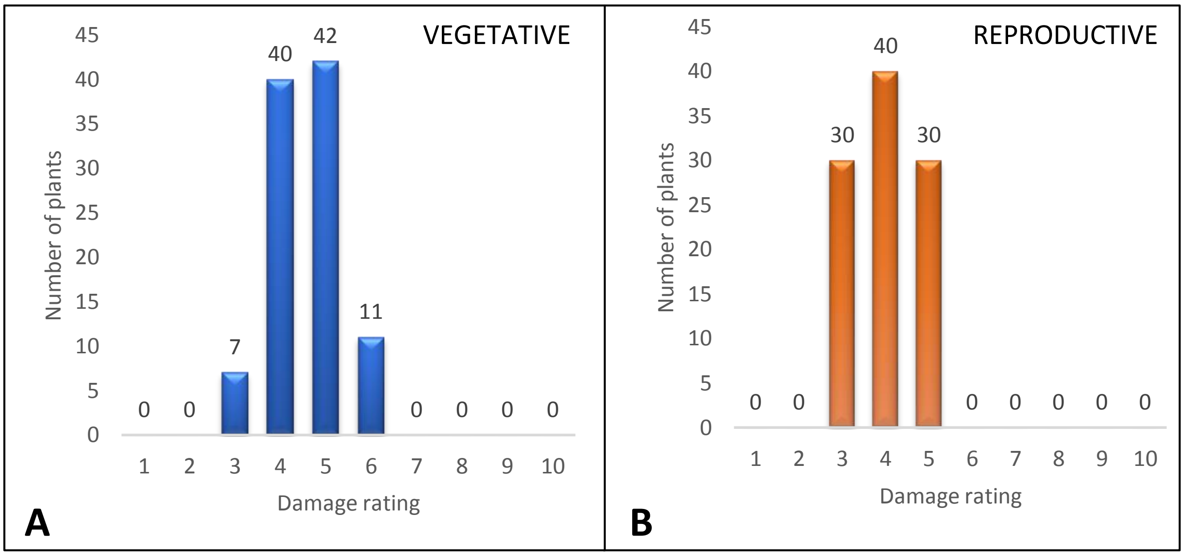

Figure 5. Distribution of representative data of damage ratings for (A) vegetative and (B) reproductive stages of corn, generated from one sampling period.

A **B**

Figure 6. Damage of Asian Corn Borer larvae on non-Bt plants: **A.** Five days after infestation (presence of pinholes, shot holes and lesions); **B**. 10 days after infestation (whorl damage) during the vegetative stage.

A **B**

Figure 7. Damage of Asian Corn Borer larvae on non-Bt plants: **A**. Five days after infestation (leaf-feeding damage on the whorl); **B**. 10 days after infestation (stalk damage) during the reproductive stage.

Table 4. Summary of Asian corn borer leaf-feeding damage ratings for corn infested at the vegetative stage. Note: All test larvae infested to Bt corn plants died and all the Bt corn plants had leaf-feeding damage rating of 1.

Location	**Day of observation**	**Mean damage rating**			**Grand mean ± SEM**	**Standard deviation**
		Rep 1	Rep 2	Rep 3		
Trial I (Dry season)						
Isabela	5 DAI	4.57	3.93	3.8	4.1 ± 0.24	0.41
	10 DAI	6.5	7.23	6.5	6.74 ± 0.24	0.42
GenSan	5 DAI	3.03	2.94	3.27	3.08 ± 0.1	0.17
	10 DAI	5.32	5.21	5.79	5.44 ± 0.18	0.31
Trial II (Wet season)						
Isabela	5 DAI	5.29	5.9	5.43	5.54 ± 0.18	0.31
	10 DAI	6.06	6.17	5.81	6.01 ± 0.11	0.18
GenSan	5 DAI	4.64	4.68	4.51	4.61 ± 0.05	0.09
	10 DAI	7.08	7.74	7.55	7.46 ± 0.2	0.34

*SEM – Standard error of the mean
*Damage rating scale (Wiseman et. al., 1966)

Highly Resistant:
0- No damage
1- Few pinholes

Resistant:
2- Several to many pinholes
3- Few shot holes & 1 or 2 elongated lesions

Intermediate:
4- Several shot holes & few elongated lesions
5- Several shot holes & elongated lesions

Susceptible:
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 very visible drying

Highly Susceptible:
9- Whorl almost eaten, plenty of elongated lesions, leaves dry & dying
10- Dying or dead plants

Damage ratings for leaf-feeding at 5 DAI for reproductive stage are summarized in Table 5. The standard deviation of plot replicates ranges from 0.03-0.48. This again indicates high precision of the ratings obtained.

Instead of leaf-feeding, stalk-feeding was assessed at 10 DAI of reproductive stage because generally larvae feed and bore into the corn stalk rather than on old leaves of the reproductive-stage corn. Still, stalk-feeding damage ratings were also highly precise as reflected by the standard deviation ranging from 0.21 to 0.71 (Table 6).

In summary, the standard deviations for damage ratings obtained at both growth stages of corn were relatively low regardless of the site of conduct, the trial, and the DAI or day of observation. Again, the computed standard deviations for all observations indicate high precision of damage ratings obtained. From this, it can be inferred that the observed uniform damage was due to the precise number of larvae delivered on each plant using the cornstalk barbecue infestation technique. Nonetheless, even if the delivery system is highly effective, larval survival and feeding is also greatly influenced by the biotic and abiotic stress factors which effect the generation of quality data. The use of net cages for field bioefficacy screening also possibly helped in preventing the negative effects of natural enemies of ACB. A good experimental methodology that accounts for the biotic and abiotic factors should be used in-tandem with the cornstalk barbeque technique to maximize precision of generated data.

Table 5. Summary of Asian corn borer leaf-feeding damage ratings of corn infested at the reproductive stage. Note: All test larvae infested to Bt corn plants died and all the Bt corn plants had damage ratings of 1 for leaf-feeding.

Location	**Observation**	**Mean damage rating**			**Grand mean ± SEM**	**Standard deviation**
		Rep 1	Rep 2	Rep 3		
Trial I (Dry season)						
Isabela	5 DAI	4	4.06	4.04	4.03 ± 0.02	0.03
GenSan	5 DAI	3.3	3.17	3.38	3.28 ± 0.06	0.11
Trial II (Wet season)						
Isabela	5 DAI	2.46	2.59	3.35	2.8 ± 0.28	0.48
GenSan	5 DAI	3.76	3.82	4.08	3.89 ± 0.1	0.17

*SEM – Standard error of the mean

*Damage rating scale (Wiseman et. al., 1966)

Highly Resistant:
- 0- No damage
- 1- Few pinholes

Resistant:
- 2- Several to many pinholes
- 3- Few shot holes & 1 or 2 elongated lesions

Intermediate:
- 4- Several shot holes & few elongated lesions
- 5- Several shot holes & elongated lesions

Susceptible:
- 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 very visible drying

Highly Susceptible:
- 9- Whorl almost eaten, plenty of elongated lesions, leaves dry & dying
- 10- Dying or dead plants

Table 6. Summary of Asian corn borer stalk-feeding damage rating of corn infested during the reproductive stage. Note: All test larvae infested to Bt corn plants died and all the Bt corn plants had damage ratings of 1 for stalk-feeding.

Location	Observation	Mean damage rating			Grand mean ± SEM	Standard deviation
		Rep 1	Rep 2	Rep 3		
Trial I (Dry season)						
Isabela	10 DAI	1.48	2.3	2.64	2.14 ± 0.34	0.60
GenSan	10 DAI	5.85	6.31	5.75	5.97 ± 0.17	0.30
Trial II (Wet season)						
Isabela	10 DAI	1.74	2.16	1.96	1.95 ± 0.12	0.21
GenSan	10 DAI	3.95	2.96	2.58	3.16 ± 0.41	0.71

SEM – Standard error of the mean
*Damage rating scale (Wiseman et. al., 1966)

Highly resistant:
0- No damage
Resistant:
<1- 1 or 2 holes above and under the corn ear
Intermediate:
1- 2 holes above and under the corn ear and broken tassel; 2 to 3 holes above and under the corn ear
Susceptible:
2-4- More than 3 hole above and under the corn ear and tassel broken
Highly susceptible:
5-9- All nodes have holes and tassel broken

Implication of the infestation technique

The cornstalk barbecue infestation technique increases the precision of the number of larvae delivered to each test plant, thereby also increasing the precision of the data gathered, particularly the damage ratings. Unlike the use of forceps, camel-hair brush, and the bazooka technique, the cornstalk barbecue technique is easier to perform during the actual field infestation, as the cornstalk barbecues need only to be pinned onto the plant. Consequently, this decreases the duration of the actual infestation. Moreover, high larval survival is another feature of this technique. Larvae, specifically second-instars, are less disturbed during the delivery as they have already bored into the cornstalks, thus minimizing direct handling of the larvae. However, the preparation of the cornstalk barbecues is labor-intensive. Nevertheless, the strengths of this technique outweigh this shortcoming. In conclusion, greater consideration should be given to this technique as an effective and efficient tool for bioefficacy testing and the generation of precise data.

Further studies are recommended to be done using other ACB larval instars. Percent recovery of infested larvae may also be obtained as an additional parameter for gauging the efficiency of this technique.

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