

## **EFFICACY OF CORN EVENT TC1507 AND THE HYBRID STACKED PRODUCT TC1507XMON810XNK603 AGAINST SECONDARY LEPIDOPTERAN PESTS OF CORN IN THE PHILIPPINES**

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### **ABSTRACT**

Field efficacy of the transgenic maize event TC1507 expressing the Cry1F protein (containing event DAS-Ø15Ø7-1), event MON810 expressing the cry1Ab protein (containing event MON-ØØ81Ø-6) and the stacked hybrid expressing Cry1F and Cry1Ab proteins (containing event DAS-Ø15Ø7-1xMON-ØØ81Ø-6xMON-ØØ6Ø3-6) against the four secondary lepidopteran pests, namely: common cutworm (CCW) *Spodoptera litura* (Fabricius), true armyworm (TAW) *Mythimna separata* (Walker), corn earworm (CEW) *Helicoverpa armigera* (Hübner), and corn semi-looper (CSL) *Chrysodeixis eriosoma* (Doubleday) was evaluated during the 2012 dry season in two regulated field trials in Cauayan, Isabela and Alcala, Pangasinan. TC1507 provided effective control against CCW, CEW and CSL, and presented a new mode of action against the primary pest, the Asian corn borer (ACB) *Ostrinia furnacalis* (Guenée). On the other hand, the triple stacked hybrid (TC1507xMON810xNK603) provided effective control against all four secondary lepidopteran pests. The combined trait product offers broader spectrum for lepidopteran pest control aside from its excellent performance against ACB.

**Key words:** Common cutworm, Corn earworm, corn hybrid, Corn semi-looper, Cry1Ab, Cry1F, True armyworm, whole plant bioassay

### **INTRODUCTION**

The introduction of Bt corn in 2003 greatly improved corn production in the Philippines. After a decade, more than 0.5 million ha. are now planted to biotech corn, expanding mainly in Luzon and Mindanao. The most outstanding benefit of Bt corn is its successful control of the primary insect pest of corn, the Asian corn borer (ACB), *Ostrinia furnacalis* (Guenée). The technology increased farm yield and farmers' income (Gonzales, 2005; Yorobe & Quicoy, 2006; Aldemita et al., 2015).

Pioneer Hi-Bred Philippines, Inc. spearheaded the hybrid corn industry in the Philippines and had developed Bt corn products for cultivation. These include Bt corn event TC1507, expressing Cry1F protein effective against ACB, and a hybrid stacked product TC1507xMON810xNK603, expressing Cry1F and Cry1Ab proteins. The latter aims for a potentially broader spectrum of lepidopteran pest control and enhanced product durability. The gene for Cry1F protein was derived from *Bacillus thuringiensis* Berliner var. *aizawai* strain PS811. This Cry1F protein is highly effective and target-specific to economically important insect pests of corn. In India, Nagaraj et al. (2007) reported that TC1507 was effective in controlling their most destructive pest of corn, *Chilo partellus* (Swinhoe), and also against the secondary pests, *Helicoverpa armigera* (Hübner) and *Spodoptera litura* (Fabricius). The work of Thompson et al. (2010) showed that TC1507 was highly effective against the Asian corn borer, *O. furnacalis* in the Philippines. It is postulated that the pyramided Bt corn hybrid will also be effective against the tropical species. However, tests still need to be conducted as part of the regulatory requirement in the Philippines.

One consequence of using Bt transgenic corn plant is that secondary pests will have the chance to become the dominant pests. Hence, it is always possible that a niche virtually vacated by greatly reduced populations of the major pest will be available for new occupants, as maybe expected in any crop-pest dynamics. The common cutworm *S. litura*, corn armyworm, *Mythimna separata* (Walker), corn earworm *H. armigera* and corn semi-looper *Chrysodeixis eriosoma* (Doubleday) are regarded as secondary pests of corn in the Philippines (Gabriel, 1997). There were already reports of sporadic lepidopteran pest outbreaks especially after prolonged droughts and flooding. In 2009, armyworm and cutworm outbreaks were observed around the country. The recent invasion of the fall armyworm *Spodoptera frugiperda* (J.E. Smith) in the country (IPCC, 2019) caused yield losses among corn farmers and these pests will likely become future threats to corn production.

A wide range of new biotech corn products are rising and these products maybe resistant or tolerant against these secondary pests. With the future threat from such pests, it is also necessary to evaluate the performance of these products. Hence, there is an urgent need to test the bioefficacy of these GM products in the field against these secondary pests to provide immediate and effective control. It is, therefore, the aims of this study to evaluate under field conditions the efficacy of TC1507 on the different secondary lepidopteran pests of corn, and to compare the efficacy of TC1507xMON810xNK603 combined event product that expresses two Bt proteins with TC1507, its single component event as well as against the single event MON810.

## MATERIALS AND METHODS

### Test and Control Plants

The test substances for the trial (Table 1) were the triple stacked Bt corn hybrid TC1507xMON810xNK603 (treatment code: T1), and the single Bt corn hybrid TC1507 (T2). The control substances were the single Bt corn hybrid MON810 (T3) and the non-transgenic isohybrid (control hybrid) (T4).

**Table 1.** Treatments and their corresponding Bt proteins used in the study

Treatment Code	Treatment Entry	Bt protein
1	TC1507XMON810XNK603	Cry1F + Cry1Ab
2	TC1507	Cry1F
3	MON 810	Cry1Ab
4	Non-transgenic control	None

### Experimental Treatments and Field Lay-out

The trial consisted of four sub-studies. Each sub-study focused on one target insect pest. Sub-studies 1-4 with target insect and insect code as common cutworm (CCW), true armyworm (TAW), corn earworm (CEW), and corn semi-looper (CSL), respectively. Each sub-study consisted of three replications per treatment in a randomized complete block design. Each treatment plot measured 6.0 m (8 rows) x 5.0m with a total experimental lot area of 3,000 m<sup>2</sup>. Seeds were planted by hand or manual seeder following a distance of 75 cm between rows and 20 cm between hills (67,000 plants per hectare). Two seeds were planted per hill and thinned out to one plant about 7-10 days after planting. A commercial Bt hybrid of the same genetic background (P3482) was planted in at least 3.0-m perimeter area surrounding the experimental lot. The treatments randomization among the experimental units unique for each site was done in Alcala, Pangasinan and Cauayan, Isabela.

### Plot Design and Insect Infestations

Each treatment plot comprised of eight rows. In each plot, rows 4 and 5 were utilized for insect evaluation (with destructive sampling) after artificial insect infestation. Rows 6 and 7 were used for insect evaluation and yield assessment following natural insect infestation. The first and last rows served as buffer rows.

The test insects were infested in five selected plants each in rows 2, 3, 4 and 5. The infested plants were selected in such a way that there were five hills between two artificially infested plants within a row. The plants selected for artificial infestation and yield data collection were tagged with colored markers as appropriate after thinning and before the scheduled infestation dates. Infested rows 4 and 5 were caged starting at insect infestation until damage scoring and larval survival data collection has been completed.

Neonate or 1<sup>st</sup> instar larvae of CCW, TAW, CEW, and CCL were manually infested twice within a 7-day period (Table 2). Insect infestations were done early in the morning or late in the afternoon to prevent the desiccation or death of test insects.

**Table 2.** The different test insects infested at a rate of 50 larvae per plant (2x) for a total of ~24,000 larvae per species in the corresponding stages of corn test plants.

Sub-study	Target insect*	Crop stage to infest	Approximate DAP
1	CCW	V4-V6	~15-25
2	TAW	V6-V9	~25-35
3	CEW	V6-V9	~25-35
		R1	~50-55
4	CSL	V4-V6	~15-25

\*CCW - Common cutworm; TAW - True armyworm; CEW - Corn earworm; CSL - Corn semi-looper

### **Management of Field Plots**

Standard agronomic practices for maize production were followed. The recommended fertilization of 120-60-90 NPK was applied consisting of a combination of basal application of complete fertilizer (eight bags) and side-dressing of urea (three bags) and muriate of potash (one bag) at 25-30 DAP per hectare basis. Hilling-up operations were done a month after planting.

No insecticide treatments were applied to the test entries, control entries nor trial borders in the two sites.

No herbicides were applied to the trial in both locations. All plots including border rows and alleyways were hand-weeded (without off-barring) at V3-V4 corn stage (~15-20 DAP). Follow-up hand weeding was conducted around 15 days after fertilizer side-dress and hilling-up (~45 DAP).

### **Statistical Analysis**

Data on percent survival and leaf damage rating were analyzed using one-way ANOVA. Treatment differences were determined using Tukey's-HSD test at 0.05 level of significance.

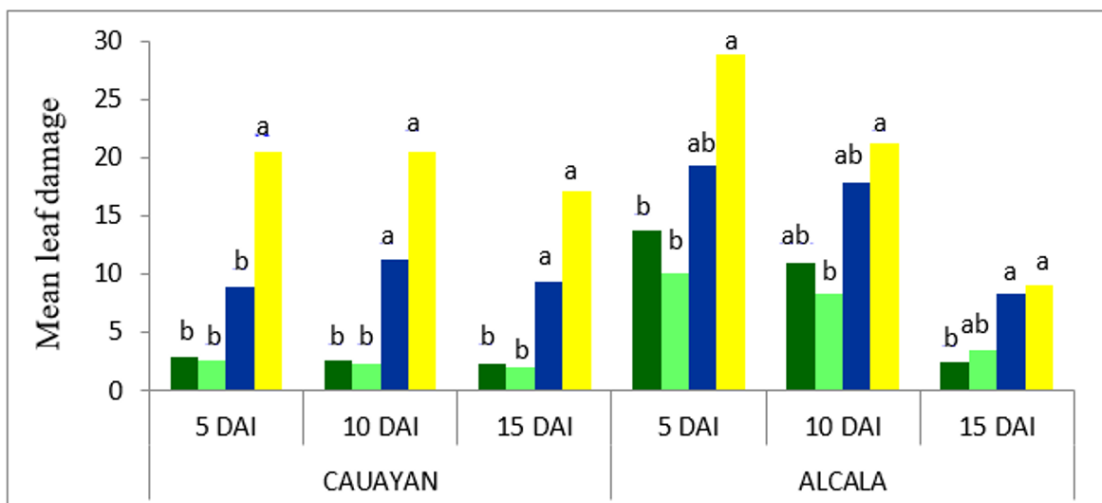
## **RESULTS AND DISCUSSION**

### **Field Efficacy of Secondary Lepidopteran Pests on Bt and Non-Bt Corn Hybrids Under Artificial Infestation**

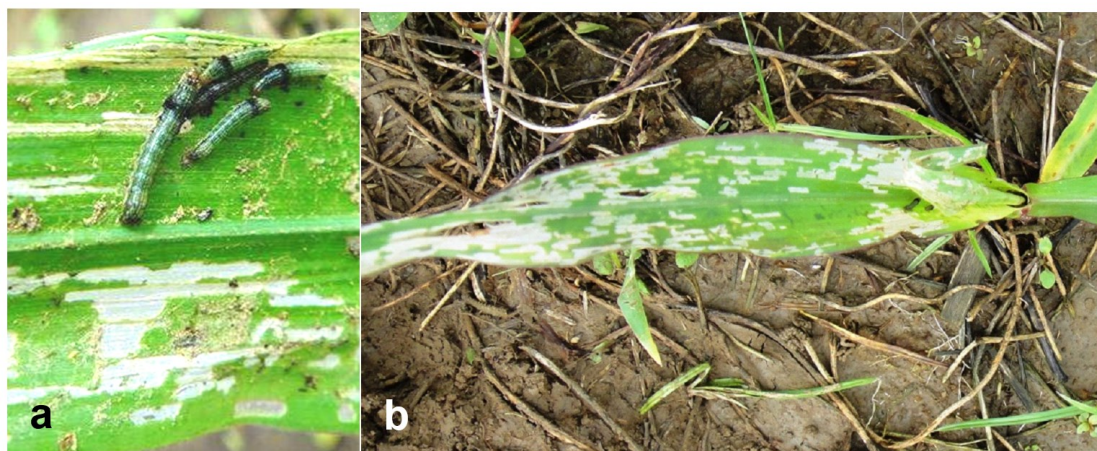
#### **Common Cutworm, *Spodoptera litura***

Mean percent leaf damage of common cutworm differed significantly among treatments at 5, 10, and 15 days after infestation (DAI) in both study sites (Figure 1). The non-transgenic conventional hybrid (T4) consistently had significantly the highest mean leaf damage ranging from 10-30%. This was followed by T3 (Bt corn MON810) with leaf damage ranging from 8.9-19%. TC1507 (T2) and its stacked hybrid (T1), TC1507xMON810xNK605, had significantly the lowest percent leaf damage especially in Cauayan ranging from 2.62-2.83% at five DAI and 1.93-2.34 at 15 DAI, respectively. Mean percent leaf damage was a little higher in Alcalá from 2.50-13.8% at 15 DAI. The higher values in Alcalá were mainly due to the greater damage by the 2<sup>nd</sup> instar larvae that were infested during the second infestation.

The study in Cauayan did not result to greater damage like the one in Alcalá because the infested insects were mostly neonates and 1<sup>st</sup> instar larvae. Nonetheless, the performances of the four treatments were consistent for all observation periods in both Cauayan and Alcalá. Both TC1507 and its stacked hybrid were resistant to the common cutworm while MON810 and the non-transgenic control hybrid were susceptible. The neonates and early instar larvae were observed to feed first on the leaves upon infestation, as shown by the scrapings on leaf surfaces (Figure 2). They continued to feed on the whorl leaves. Then, the third instar and maturing larvae directly fed on the stem near the tip portion defoliating the plant and eventually cutting off the main stem. The plants would eventually die when the growing tip had been eaten.



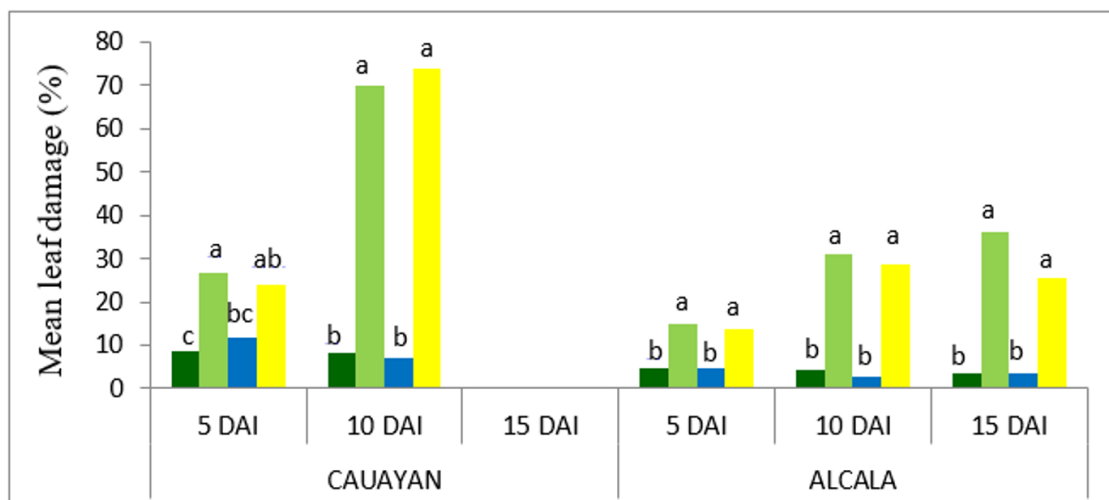
**Figure 1.** Mean leaf damage (%) of common cutworm on 10 randomly selected test plants of Bt and non-Bt corn hybrids 5, 10, and 15 days after infestation (damage assessment per plant basis on rows 4 and 5 (caged plants) in Cauayan, Isabela and Alcala, Pangasinan. T1 ■ TC1507xMON810xNK603; T2 ■ TC1507; T3 ■ MON810; T4 ■ Non-transgenic control hybrid.



**Figure 2.** Damage of common cutworm (*Spodoptera litura*) on 18-day old corn plant. **a.** Neonates and early instar larvae initially feed on the leaves. **b.** Larvae continue to feed on the stem near the tip portion, defoliating the plant, and eventually cutting off the main stem.

### **True armyworm, *Mythimna separata***

Significant differences were observed in leaf damage levels of the true armyworm among the four treatments (Figure 3). Mean leaf damage in Cauayan was significantly highest on non-transgenic control. The damage on TC1507 at five DAI and 10 DAI were the same as that in the non-transgenic control hybrid in both sites. At 15 DAI, the plants were already damaged when dissected. The



**Figure 3.** Mean leaf damage (%) of true armyworm on 10 randomly selected test plants of Bt and non-Bt corn hybrids 5, 10, and 15 days after infestation (damage assessment per plant basis on rows 4 and 5 - caged plants) in Cauayan, Isabela and Alcala, Pangasinan. T1 ■ TC1507xMON810xNK603; T2 ■ TC1507; T3 ■ MON810; T4 ■ Non-transgenic control hybrid.

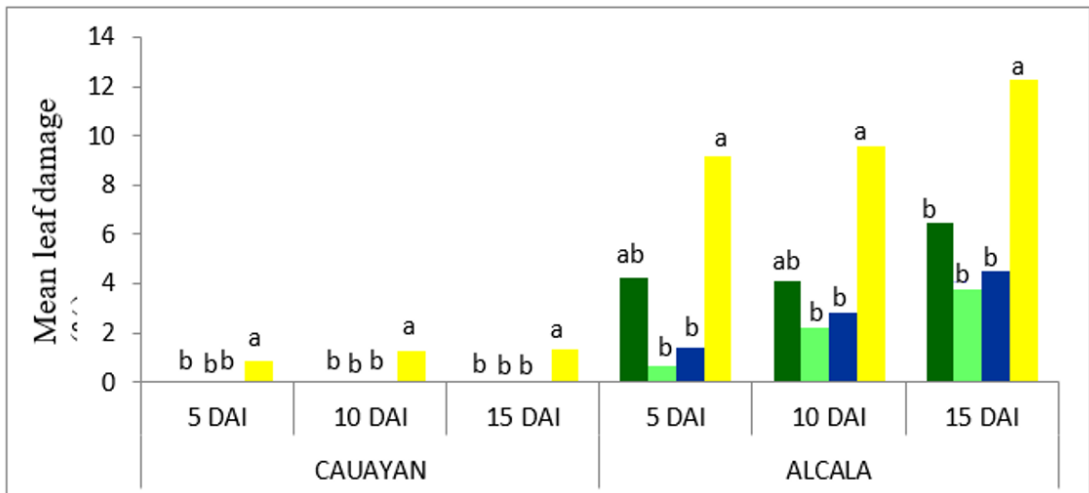
strong winds from the typhoon during the infestation period in Alcala, Pangasinan had affected the test insects and only few larvae survived as reflected by the generally lower percent leaf damage for TC1507 and the non-transgenic conventional control hybrid (ranging from 12-30%). However, despite this setback, percent damage among treatments still showed significant differences in both locations. The triple stacked hybrid (TC1507xMON810xNK603) and MON810, had significantly lower percent leaf damage and the values were consistently low for all observations periods. Hence, these two Bt corn hybrids were resistant to the true armyworm. On the other hand, TC1507 and non-transgenic conventional control hybrid were susceptible. The newly infested larvae initially bore into the whorl and continued feeding (Figure 4). Once they mature, they transfer to nearby fully expanded leaves and started feeding on the leaf margins until they consume everything, leaving the midribs and the main stalk.

### **Corn earworm, *Helicoverpa armigera***

Results for the percent leaf damage by the corn earworm was considered only in Alcala. The larvae that were infested in Cauayan did not establish well due to inclement weather during the infestation period. In Alcala, there was a significant difference in the leaf damage by the corn earworm on the different treatments (Figure 5). The damage on TC1507 (T2) and MON810 (T3) ranged from 0.8-3.0% as compared to 8-12% for the non-transgenic control (T4) and the stacked hybrid (T1) with 4-6%. Overall, leaf damage was significantly less for Bt corn hybrids compared to the non-transgenic control. Under natural field conditions, the damage of earworm on the leaves is usually concentrated on the



**Figure 4.** Damage of true armyworm (*Mythimna separata*) on the corn plant. **a.** before infestation. **b.** caged plants showing leaf feeding damage. **c.** heavily damaged non-transgenic conventional plants. **c-d.** distinct characteristic feeding patterns of true armyworm larvae on the edges of the leaves.



**Figure 5.** Mean leaf damage (%) of corn earworm on 10 randomly selected test plants of Bt and non-Bt corn hybrids 5, 10, and 15 days after infestation in Cauayan, Isabela and Alcala, Pangasinan. T1 ■ TC1507x-MON810xNK603; T2 ■ TC1507; T3 ■ MON810; T4 ■ Non-transgenic control hybrid.

whorl (Figure 6). Once the larvae got older, they bore and feed deeper into the whorl. As the leaves expanded, the holes become visible due to previous larval feeding.



**Figure 6.** Damage of corn earworm (*Helicoverpa armigera*) on the whorl. **a.** Plants before infestation. **b-c.** Leaves showing feeding holes 15 days after infestation.

#### **Corn semi-looper, *Chrysodeixis eriosoma***

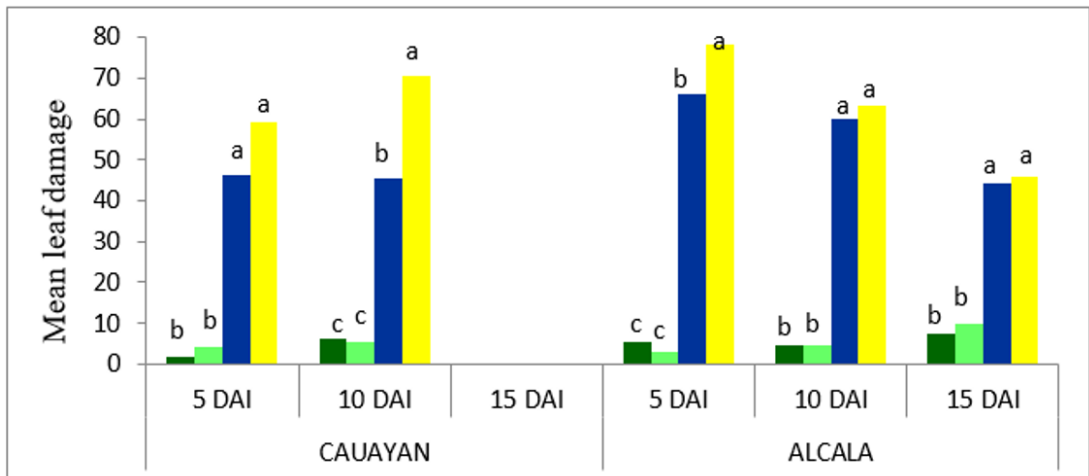
Leaf feeding damage by the corn semi-looper was significantly lower (2-6%) on both T1 (Triple stacked) and T2 (TC1507) but significantly higher on T3 (MON810) and T4 (non-transgenic control hybrid), ranging from 40-80% (Figure 7). Results for all the treatments were consistent in all observation periods and in both Cauayan and Alcala. No observations were made at 15 DAI in Cauayan as the plants in the conventional plots were already severely damaged (Figure 8). The damage caused by the corn semi-looper on the conventional hybrids initially started at the lower leaves. They fed or scraped the lower epidermis of these older leaves uniquely leaving lesions with the fine (upper epidermal) film and veins. They concentrated their feeding on the inner part of the leaves and formed holes once the damaged parts dried up.

Under severe infestation, whole leaves are consumed leaving only veins and midribs. Also, larvae did not feed on the whorl. Overall, results revealed that TC1507 and the triple stacked hybrid were resistant to the corn semi-looper. In contrast, MON810 and the non-transgenic control hybrid were susceptible.

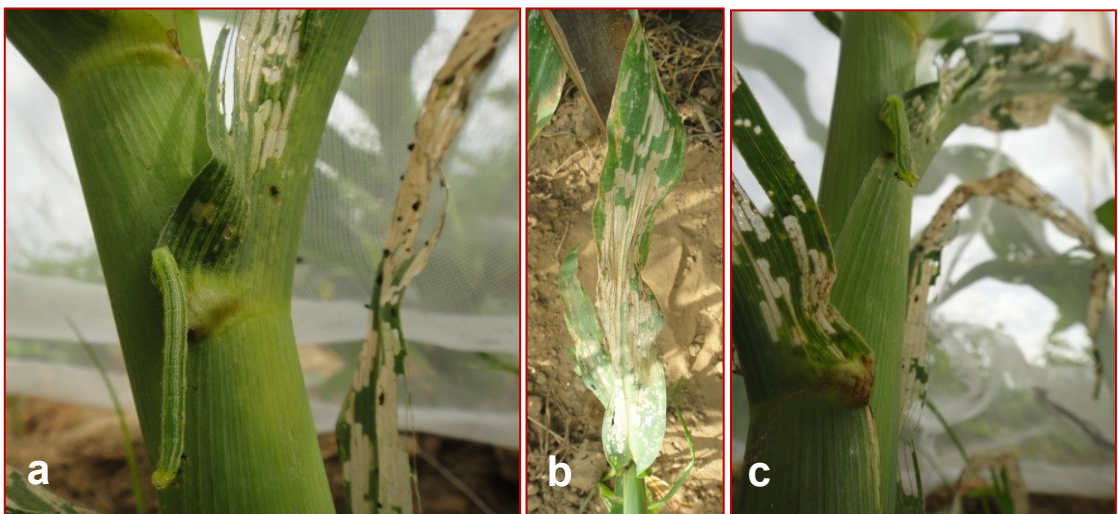
#### **Number of Surviving Larvae**

The larvae collected from the 10 test plants (destructive sampling) for larval staging (i.e., determining the stage of surviving larvae) were recovered from true armyworm and corn semi-looper plots. There were no more larvae recovered from plots infested with the common cutworm and corn earworm.

The mean number of surviving larvae was significantly highest on the non-transgenic hybrid (T4) reaching 152 larvae followed by those collected from TC1507 with 126 true armyworm larvae (Table 3) in Cauayan site. Only 8.67 and 5.67 larvae were collected from T1 (Triple stacked hybrid) and T3 (MON810), respectively. There were fewer larvae collected from Alcala because of the effect of typhoon during the day of insect infestation. However, the same results were



**Figure 7.** Mean leaf damage (%) of corn semi-looper on 10 randomly selected test plants of Bt and non-Bt corn hybrids 5, 10, and 15 days after infestation Cauayan, Isabela and Alcala, Pangasinan. T1 ■ TC1507xMON810xNK603; T2 ■ TC1507; T3 ■ MON810; T4 ■ Non-transgenic control hybrid.



**Figure 8.** Damage of corn semi-looper (*Chrysodeixis eriosoma*) on corn. **a.** Leaves of conventional hybrid already damaged at 5 DAI leaving a thin film of the upper epidermis and the veins. **b-c.** leaf feeding progressing at 10 DAI.

obtained with almost no larvae collected from the triple stacked hybrid and MON810. This confirms the previous result that TC1507 was susceptible to the true armyworm and that the Cry1F protein is not effective in controlling this lepidopteran pest.

Significantly more surviving larvae of the corn semi-looper were observed on MON810 and on non-transgenic control hybrid in Cauayan, Isabela. There

**Table 3.** Mean number of surviving larvae of the four lepidopteran pests of corn dissected from 10 caged plants with artificial infestation on Bt and non-transgenic control hybrids in Alcala, Pangasinan and Cauayan, Isabela.

Site	Treatments	Number of surviving larvae*			
		Common cutworm	True armyworm	Corn Earworm	Corn semi-looper
<b>Alcala Pangasinan</b>	T1 - Triple Stack	0.00 a	0.33 b	1.33 a	0.00 a
	T2- TC1507	0.00 a	8.00 a	3.67 a	0.33 a
	T3- MON810	0.00 a	0.00 b	1.67 a	0.67 a
	T4- non-transgenic Conventional	0.67 a	5.33 ab	4.00 a	1.00 a
<b>Cauayan Isabela</b>	T1 - Triple Stack	0.00 a	8.67 b	0.00 a	0.00 b
	T2- TC1507	0.00 a	126 a	0.00 a	0.00 b
	T3- MON810	0.00 a	5.67 b	0.00 a	9.00 a
	T4- non-transgenic Conventional	0.00 a	152.00 a	0.00 a	15.70 a

\*Values are means of 3-plot replications. Larvae were collected from 10 caged plants in various treatments 10 days after artificial insect infestation. Means followed by a common letter are not significantly different using Tukey's-HSD at  $\alpha = 0.05$ .

was no significant difference in the number of semi-loopers in Alcala, Pangasinan. Interestingly, no larvae survived on the triple stacked hybrid and TC1507. Thus, MON810 and the non-transgenic control hybrid are susceptible to the corn semi-looper. Cry1Ab protein on MON810 does not provide effective control against this corn pest.

### Comparative Efficacy of Bt and Non-Bt Corn Hybrids Against the Secondary Lepidopteran Pests

Table 4 compares the efficacy of the different treatments: T1 (Triple stacked), T2 (TC1507), T3 (MON810) and T4 (Non-transgenic\_control hybrid) against the four secondary lepidopteran insect pests based on overall ranking using the data on mean percent leaf damage, and designating a qualitative rating of resistant, intermediate, and susceptible.

The triple stacked hybrid (TC1507xMON810xNK603) carrying the Cry1Ab and Cry1F proteins was resistant to the three lepidopteran pests (common cutworm, true armyworm, and corn semi-looper), and intermediate to corn earworm. TC1507 with Cry1F protein was resistant to the common cutworm and corn semi-looper but susceptible to the true armyworm. TC1507 was resistant to leaf feeding of the corn earworm. On the other hand, the Bt corn hybrid MON810 carrying the Cry1Ab protein was resistant to the true armyworm but susceptible to common cutworm and corn semi-looper. MON810 was moderately resistant to the corn earworm.

Table 5 summarizes the potential of the single and pyramided hybrids. TC1507 containing the Cry1F protein has provided effective control against the leaf feeding of the three lepidopteran pests: common cutworm, corn semi-looper and corn earworm. On the other hand, MON810 containing the Cry1Ab protein has provided effective control on leaf feeding by true armyworm and corn earworm. Combining the two proteins together in the triple stacked hybrid have provided greater control against all the four lepidopteran pests including

**Table 4.** Comparative field efficacy of TC1507 and its stacked hybrid (TC1507 x MON810 xNK603) and non-transgenic corn hybrid against four secondary lepidopteran pests based on overall ranking using mean percent leaf damage and designating a qualitative rating of resistant, intermediate, and susceptible. Pooled data from the two field trials in Cauayan, Isabela and Alcala, Pangasinan (2012 dry season).

Treatment code and entry	Bt Cry protein	Common Cutworm (leaf)	True armyworm (leaf)	Corn earworm (leaf)	Corn semi-looper (leaf)
T1-Triple stack	Cry1Ab + Cry1F	R	R	I	R
T2-TC1507	Cry1F	R	S	R	R
T3-MON810	Cry1Ab	MS	R	MR	S
T4-non-transgenic Conventional	None	HS	HS	HS	HS

<sup>a</sup>R- Resistant; I- Intermediate; S- Susceptible; HS- Highly Susceptible

**Table 5.** Overall field performance of TC1507, MON810 and its stacked hybrid (TC1507xMON810xNK603) and non-Bt corn hybrid against the four secondary lepidopteran pests using pooled data from the two field trials in Cauayan, Isabela and Alcala, Pangasinan (2012 dry season).

Treatment Code	Treatment Entry	Bt protein	Efficacy Against Secondary Lepidopteran Pests
1	TC1507x MON810x NK603	Cry1F + Cry1Ab	Common cutworm True armyworm Corn earworm (leaf) Corn semi-looper
2	TC1507	Cry1F	Common cutworm Corn earworm (leaf) Corn semi-looper
3	MON 810	Cry1Ab	True armyworm, Corm semi-looper (silk)
4	Non-transgenic control	None	None

resistance to silk damage by the corn earworm. The results clearly demonstrate the advantage of combining the two proteins for effective management of secondary lepidopteran pests (Table 5). The performance of the hybrids, i.e., varietal reaction to lepidopterous pests, is summarized in Table 6.

The combination or stacking of traits in a product was better than the two single trait products implying that the two proteins are complementary.

There is dual mode of action, with TC1507xMON810xNK603 expressing both Cry1F and Cry1Ab proteins. Together, these two have shown to be many folds more durable compared to the single events expressing Cry1Ab or Cry1F alone (Tan, et al 2013; Wang et al 2016).

This study also confirmed under our tropical conditions the previous results in other countries like the US, that this combined trait product will potentially offer broader spectrum lepidopteran pest control. This is on top of the excellent performance of this new enhanced stacked Bt corn against the Asian corn borer (Caasi-Lit, 2017). To reiterate, this stacked product is expected to

**Table 6.** Summary reaction of different Bt corn hybrids to secondary lepidopteran pests as compared to the Asian corn borer.

<b>Lepidopteran Pests</b>	<b>TC1507</b>	<b>MON810</b>	<b>TC1507xMON810xNK603</b>
Common cutworm	Very good	Moderate	Very good
True armyworm	Poor	Very good	Very good
Corn earworm (leaf)	Moderate	Good	Very good
Corn semi-looper	Excellent	Poor	Excellent
Asian corn borer*	Excellent <sup>a</sup>	Excellent <sup>b</sup>	Excellent <sup>c</sup>

\*From previous results: a - Thompson *et al.* 2010; b - Fernandez et al. 1997; c - Caasi-Lit 2017.

enhance product stability and is now fully recommended for commercial production. The approval of this Bt corn product by the Bureau of Plant Industry, the official regulatory agency in the Philippines, is part of a regulatory procedure for IRM for Bt corn testing and commercialization. This is also timely when secondary lepidopteran pests are becoming potential threats to corn production.

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