*Journal of Economic Entomology*, 113(2), 2020, 974–979
doi: 10.1093/jee/toz367
Advance Access Publication Date: 22 January 2020
Research



# Efficacy of a *cry1Ab* Gene for Control of *Maruca vitrata* (Lepidoptera: Crambidae) in Cowpea (Fabales: Fabaceae)

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Subject Editor: Frank Peairs

Received 26 September 2019; Editorial decision 20 December 2019

## Abstract

Cowpea [*Vigna unguiculata* (L) Walp.] is an important staple legume in the diet of many households in sub-Saharan Africa. Its production, however, is negatively impacted by many insect pests including bean pod borer, *Maruca vitrata* F., which can cause 20–80% yield loss. Several genetically engineered cowpea events that contain a *cry1Ab* gene from *Bacillus thuringiensis* (Bt) for resistance against *M. vitrata* were evaluated in Nigeria, Burkina Faso, and Ghana (West Africa), where cowpea is commonly grown. As part of the regulatory safety package, these efficacy data were developed and evaluated by in-country scientists. The Bt-cowpea lines were planted in confined field trials under Insect-proof netting and artificially infested with up to 500 *M. vitrata* larvae per plant during bud formation and flowering periods. Bt-cowpea lines provided nearly complete pod and seed protection and in most cases resulted in significantly increased seed yield over non-Bt control lines. An integrated pest management strategy that includes use of Bt-cowpea augmented with minimal insecticide treatment for protection against other insects is recommended to control pod borer to enhance cowpea production. The insect resistance management plan is based on the high-dose refuge strategy where non-Bt-cowpea and natural refuges are expected to provide *M. vitrata* susceptible to Cry1Ab protein. In addition, there will be a limited release of this product until a two-toxin cowpea pyramid is released. Other than South African genetically engineered crops, Bt-cowpea is the first genetically engineered food crop developed by the public sector and approved for release in sub-Saharan Africa.

**Key words:** transgenic, genetically engineered Bt-cowpea, West Africa

Cowpea [*Vigna unguiculata* (L) Walp.], also known as black-eyed pea, is an important staple in the diet of more than 200 million households in sub-Saharan Africa (Kamara et al. 2016). Cowpea is a resilient legume that withstands low rainfall and poor soil conditions of the semi-arid and subhumid areas of the region and has the ability to fix soil nitrogen (Boukar et al. 2015). Its protein-rich seeds provide valuable nutrition to humans including its use as an important weaning food for human babies (Bassey et al. 2013). Unlike many other legumes, cowpea's green leaves and immature pods also are edible (Bressani 1985). Perhaps because of its high nutritive value, cowpea has many insect pests (Jackai and Daoust 1986). *Maruca vitrata* F., known as the bean pod borer or legume pod borer, is among the major insect pests of cowpea (Taylor 1967, Jackai and Daoust 1986, Kamara et al. 2007, Ba et al. 2019). Larvae of this pest infest the reproductive organs, including flower buds, flowers, and pods. Cowpea yield losses due to this pod borer range from 20 to

80% (Singh and van Emden 1979, Ba et al. 2019). Presently, there are no known cowpea varieties resistant to *M. vitrata*; hence, farmers regularly spray insecticides five to eight times within a season to control this and other insect pests (Jackai and Adalla 1997, Murdock et al. 2008). Traditional insecticides, however, are not typically effective against *M. vitrata* larvae after they bore into the cowpea floral parts and pods. The inability to control these larvae is a serious challenge to cowpea production and its availability as food (Ekesi 1999, Abudulai et al. 2017, Ba et al. 2019).

Genes for insecticidal proteins from *Bacillus thuringiensis* (Bt) expressed in plants such as maize, cotton, eggplant (brinjal), and soybean are highly effective for insect control, which has led to the rapid adoption and commercialization of Bt protected crops in many developed and developing countries (James 2014, Naranjo et al. 2020). These crops provide effective control of major lepidopteran insect pests such as the European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae), Old World bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), and fruit and shoot borer, *Leucinodes orbonalis* (Guenée) (Lepidoptera: Crambidae). This has led to reduced reliance on conventional pesticides for the protection of those crops against these insects, thus reducing costs from pesticide purchase and use, and improving product safety to farmers and consumers as well as providing higher yields in each crop (Klümper and Qaim 2014, Brookes and Barfoot 2018). Like the Bt eggplant project in Bangladesh, Bt-cowpea can be an important development for resource-poor farmers in Africa (Shelton et al. 2018).

A Bt *cry1Ab* gene, similar to that used in maize event MON 810, was used to develop the genetically engineered (GE) Bt-cowpea for small-holder farmers in sub-Saharan Africa (Higgins et al. 2012). In laboratory studies, Cry1Ab toxin is highly toxic against *M. vitrata* early instars (Srinivasan 2008). The objective of this study was to evaluate efficacy of the *cry1Ab* gene in Bt-cowpea for control of *M. vitrata*. Other than South African GE crops, Bt-cowpea is the first GE food crop developed by the public sector and approved for release in sub-Saharan Africa. One of the Bt-cowpea events described in this paper, 709A, was introgressed into the farmer-preferred variety, SAMPEA 10 and will be marketed to farmers as Pod Borer Resistant (PBR) Cowpea (PBR Cowpea). The use of 'Bt-cowpea' herein is a simpler description of the material evaluated.

## Materials and Methods

The *cry1Ab* gene was introduced into the cowpea line IT86D-1010 by *Agrobacterium*-based transformation (Popelka et al. 2006, Higgins et al. 2012) at the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Canberra, Australia to generate a large collection of isogenic GE events for screening. Each of the Bt-cowpea events was screened for a single copy of the transgene, absence of vector backbone sequences, and for homozygosity. Seeds of selected events were produced for evaluation in confined field trials (CFT). Briefly, a CFT is a field trial of a GE crop performed by researchers prior to commercial approval for cultivation so that they are able to safely evaluate the crop with new genetic traits (Halsey 2006). Regulation, conduct, and oversight of CFT are conducted by host country regulators. For this project, GE events were screened in CFTs in Puerto Rico between 2007 and 2009, and in Nigeria in 2009 and 2010. The GE events 162B, 212D, 252D, 709A, 721C, 1011E, and the negative non-Bt control IT86D-1010, isoline with each of the events, were selected and planted at research farms associated with the Institute for Agricultural Research (IAR) and Ahmadu Bello University (ABU) at Zaria, Nigeria, and Institut de l'Environnement et de Recherches Agricoles (INERA) at Farako Ba, Burkina Faso in 2011. Another set of events including 252D, 709A, 1023A, 1023C, 1023G, 1023J, and non-Bt control IT86D-1010 (isoline) were planted and evaluated at IAR, Nigeria and INERA, Burkina Faso in 2012. The event 709A was identified as an elite event that was very efficacious in preventing *M. vitrata* plant injury, and hybrids formed from this event also were efficacious (Mohammed et al. 2014). Event 709A was introgressed into a cowpea variety preferred by farmers, SAMPEA 10 (original name IT97K-499-35) through conventional breeding. This generated the PBR Cowpea (PBRC). The non-Bt line (PBC) was generated as null segregants derived from PBRC during the backcrossing process to produce a non-Bt near-isoline cowpea. Real-time polymerase chain reaction (PCR) and progeny testing in the next generation were used to identify null segregants (Schmidt and Parrott 2001). Event 709A was renamed event AAT709A for the regulatory submission in Nigeria (NBMA 2019).

### Experimental Design

In 2011, six Bt-cowpea events, 162B, 212D, 252D, 709A, 721C, 1011E, and the non-Bt control IT86D-1010 isoline were planted in CFT in August in a randomized complete block design with three replications at each of the research farms in Nigeria (IAR/ABU) and Burkina Faso (INERA). These farms are located in Guinea Savanna (Nigeria) and Sudan Savanna (Burkina Faso) agro-ecological zones in sub-Saharan Africa. Each plot comprised two rows 3.0 m long. The seeds were planted at 0.20 m intervals within rows and 0.75 m between rows. Each plot consisting of 30 plants was separated by 1.5 m of unplanted walkways. Similarly, 1.5 m of unplanted paths separated the replicates. The plants were checked for the presence or absence of the *cry1Ab* gene by using Gene Check ELISA (enzyme-linked immunosorbent assay) based kits produced by EnviroLogix Company, USA, (product No.AS003 CRLS). All plants were in an enclosed area covered with a 0.6 × 0.6 mm hole size mesh netting obtained from Mosquito Curtains Inc. USA (Product Name: No-see-um) to restrict entry of other insect pests. The fertilizers NPK (15-15-15) and single superphosphate (18% P2O5) were applied at recommended rates (Dugje et al. 2009). The trial was kept weed-free by manual weeding.

In 2012, the Bt-cowpea events, 252D, 709A, 1023A, 1023C, 1023G, 1023J, and the non-Bt control IT86D-1010 isoline were tested in CFT at the same two research farms. Similar experimental design and numbers of replications (3), as well as experimental procedures, were used at each location as in 2011.

In 2015, the Bt-cowpea line PBRC and its near-isogenic non-Bt line, PBC were planted in CFT at the research farms in Nigeria (IAR/ABU) and Burkina Faso (INERA). Additionally, a confined field trial with the same Bt and non-Bt lines was established at the Savanna Agricultural Research Institute (SARI) research farm at Tamale, Ghana in the Guinea Savanna agro-ecological zone. The seeds were planted in a randomized complete block design with four replications in August at both IAR/ABU and INERA locations and in November at the SARI location. The SARI trial in Ghana was planted 3 mo late because of regulatory delays. Experimental procedures at each location were similar to those used in 2011 and 2012, except four replicates were established. In 2016, another Ghana confined field trial was established at SARI in August, the normal planting time.

### Source of Insects and Infestation Methods

*Maruca vitrata* larvae used for the trails were obtained from mass-rearing facilities at IAR/ABU, INERA, and SARI. The insect colonies were established from *M. vitrata* moths collected from light traps in each country. The adults were put in cups for oviposition in a room maintained at 24°C with 58–75%RH. Larvae were reared on an artificial diet modified from methods developed by Jackai and Raulston (1988). In this case, larvae were maintained on a modified *O. nubilalis* diet obtained commercially from Bio-Serv Company, Flemington, NJ (Bio-Serv product No. F9478B-M, without corncob grits) supplemented with cowpea flour. Subsequent generations were obtained after 24 d under the above-mentioned conditions. The cowpea plants were infested with neonates (<24 h) harvested from the laboratory early in the morning. A small brush was used to apply neonates to plants. In 2011 and 2012, 20 neonates of *M. vitrata* were used to artificially infest each plant at the onset of flower bud formation that occurred about 30 d after planting. Infestations were repeated weekly for 5 wk.

In 2015 and 2016, 50 neonates of *M. vitrata* were used to infest each plant at the onset of flower bud formation. High insect pressure was obtained by artificially infesting all the Bt-cowpea and non-Bt-cowpea plants at 3-d intervals, totaling 10, 14, and 16 infestations during the season at the SARI, IAR/ABU, and INERA locations, respectively.

### Data Collection

Days from planting to first flower was recorded for each plant in 2011, 2012 and for each plot in 2015 and 2016. Pods damaged by *M. vitrata* were identified by holes made by larvae on individual pods. Other parameters measured included plant height (soil level to top of plant excluding tendrils), total pods produced per plant, and number of pods damaged by *M. vitrata* per plant. These measurements were used to calculate percentage of *M. vitrata* pod damage per plant. Total seed weight and healthy seed weight per plant also were determined.

### Statistical Analysis

An analysis of variance (ANOVA) was conducted with the mixed linear model procedure, PROC MIXED in SAS (SAS 9.4, SAS Institute Inc., Cary, NC). The Kenward-Rogers approximation was used to account for different size variance components in the model. SAS, by default, pools the error degrees of freedom into the overall error when a random effect in the model has an estimated zero variance. Within the context of the ANOVA, Dunnett's test was used to compare each test event to the control event.

The statistical model used was:

$$y_{ijkl} = \mu + C_i + B(C)_{ij} + E_k + (CE)_{ik} + P(B)_{jl} + e_{ijkl}$$

where

$y_{ijkl}$ = measurement of plant l from block j for event k in country i
$\mu$ = overall mean
$C_i$ = Effect of country i
$B(C)_{ij}$ = Effect of block j within country i
$E_k$ = Effect of event k
$(CE)_{ik}$ = Interaction of country i with event k
$P(B)_{jl}$ = Effect of plant l within block j
$e_{ijkl}$ = residual experimental error
$C_i$ $B(C)_{ij}$ $(CE)_{ik}$ $P(B)_{jl}$ and $e_{ijkl}$ are random effects; $E_k$ is a fixed effect.

In some situations, the above model was not applicable. In 2015, the *M. vitrata* pod damage across country variability could not be stabilized. Instead, the data were first averaged over plants and then averaged over the three countries. The analysis was an analysis of variance with the PBRC and PBC means compared using Welch's test for means with unequal variances. In 2015, first flowering measurements were averaged over the plants, which necessitated the removal of the plant within block effect from the model. Except for first flowering, the model for Ghana in 2016 was a randomized complete block design with four blocks and 30 plants within each block. The statistical model was:

$$y_{ijk} = \mu + B_i + E_j + (BE)_{ij} + P(B)_{ik} + e_{ijk}$$

where

Yijk = measurement of plant k from block i for event j
μ = overall mean
$B_i$ = Effect of block i
$E_j$ = Effect of event j
$(BE)_{ij}$ = Interaction of block i with event j
$P(B)_{ik}$ = Effect of plant k within block i
$e_{ijk}$ = residual experimental error
$B_i$, $(BE)_{ij}$, $P(B)_{ik}$ and $e_{ijk}$ are random effects; $E_j$ is a fixed effect

For first flowering, the measurements were averaged over plants and the plant within block effect was removed from the model.

## Results

For Nigeria and Burkina Faso in 2011 (Table 1), the plant height ANOVA was not statistically significant ($F_{6,6}$= 1.32, $P$ = 0.3723). There also were no significant differences for days to first flowering ($F_{6,6.01}$ = 2.19, $P$ = 0.1810). There were differences in the number of pods produced per plant ($F_{6,6}$ = 6.92, $P$ = 0.0165); but only events 252D and 709A produced significantly more pods than the non-Bt control plants ($P$ = 0.0215, 0.0126, respectively). Percent pod damage caused by the *M. vitrata* was significantly lower in all the Bt events than the non-Bt control plants ($F_{6,6}$ = 6.4, $P$ = 0.0200). Their total ($F_{6,6.7}$ = 3.44, $P$ = 0.0689) and healthy seed weights per plant ($F_{6,6.67}$ = 3.63, $P$ = 0.0617) were not significantly higher than the non-Bt control; however, results from statistical contrasts suggest events 252D and 709A were near exceptions or exceptions: total seed weights per plant 252D ($P$ = 0.0561), 709A ($P$ = 0.0277); healthy seed weights per plant 252D ($P$ = 0.0427), 709A ($P$ = 0.0206).

For Nigeria and Burkina Faso in 2012 (Table 2), the plant height ANOVA was not statistically significant ($F_{6,5.74}$ = 3.55, $P$ = 0.0783). Results from statistical contrasts, however, suggest events 1023C ($P$ = 0.0332) and 709A ($P$ = 0.0357) were taller than the non-Bt control. The ANOVA for days to first flowering was not statistically significant ($F_{6,5.89}$ = 1.33, $P$ = 0.3703). All the Bt plants produced more pods per plant than the non-Bt control plants ($F_{6,5.92}$ = 9.12, $P$ = 0.0086). Percent pod damage by *M. vitrata* was significantly lower for all the Bt lines compared with the non-Bt control ($F_{6,6}$ = 108.79, $P$ < 0.0001). As a result, ANOVAs for both total seed weight per plant ($F_{6,5.95}$ = 8.40, $P$ = 0.0104) and healthy seed weight per plant ($F_{6,5.87}$ = 9.79, $P$ = 0.0074) were significantly different, as Bt-cowpea weights were all higher than those of the non-Bt controls.

For Nigeria, Burkina Faso and Ghana in 2015 (Table 3), the Bt line PBRC developed from event 709A was not significantly different from its near-isogenic non-Bt line PBC in plant height ($F_{1, 2.01}$ = 0.06, $P$ = 0.8351) and days to first flowering ($F_{1, 2}$ = 1.02, $P$ = 0.4190). Bt-cowpea produced significantly more pods per plant, ($F_{1, 3.59}$ = 11.07, $P$ = 0.0344), with significantly less pod damage ($F_{1, 3.02}$ = 777.98, $P$ < 0.0001); however, total ($F_{1, 3.97}$ = 4.96, $P$ = 0.0904) and healthy ($F_{1, 4.01}$ = 5.88, $P$ = 0.0722) seed weights,

**Table 1.** Nigeria and Burkina Faso 2011: Evaluation of cowpea Bt events and their non-Bt (NBt) control (IT86D-1010) with means for plant height, days to first flowering, total pods per plant, percent pods with *M. vitrata* pod damage, total and healthy seed weights per plant; overall ANOVA statistics with SE, and results of six contrasts (each Bt event compared with non-Bt control) for each variable

| Bt Events/ Non-Bt Control | Plant height (cm) | Days to first flowering | Total pods/plant | *M. vitrata* pod damage (%) | Total Seed weight/plant (g) | Healthy Seed weight/plant (g) |
|---|---|---|---|---|---|---|
| 162B | 22.0 | 45.3 | 9.8 | 2.8* | 16.6 | 16.3 |
| 212D | 19.0 | 42.6 | 17.0 | 1.1* | 22.7 | 22.1 |
| 252D | 22.3 | 44.4 | 19.0* | 1.1* | 28.7 | 27.8* |
| 709A | 21.4 | 43.4 | 20.2* | 0.7* | 31.6* | 31.1* |
| 721C | 22.3 | 43.0 | 15.4 | 2.4* | 22.8 | 22.5 |
| 1011E | 19.0 | 42.5 | 14.9 | 4.6* | 23.3 | 22.7 |
| NBt Control | 17.9 | 46.3 | 9.5 | 58.3 | 12.1 | 8.5 |
| SE | 5.0 | 2.3 | 2.4 | 9.4 | 3.8 | 4.0 |
| Pr > F | 0.3723 | 0.1810 | 0.0165 | 0.0200 | 0.0689 | 0.0617 |

Contrasts statistics: * $P \leq 0.05$.

**Table 2.** Nigeria and Burkina Faso 2012: Evaluation of cowpea Bt events and their non-Bt (NBt) control (IT86D-1010) with means for plant height, days to first flowering, total pods per plant, percent pods with *M. vitrata* pod damage, total and healthy seed weights per plant; overall ANOVA statistics with SE, and results of six contrasts (each Bt event compared with non-Bt control) for each variable

| Bt Events/ Non-Bt Control | Plant height (cm) | Days to first flowering | Total pods/plant | *M. vitrata* pod damage (%) | Total seed weight/plant (g) | Healthy seed weight/plant (g) |
|---|---|---|---|---|---|---|
| 252D | 32.2 | 42.0 | 13.8* | 4.4** | 21.7* | 20.3* |
| 709A | 34.3* | 40.3 | 12.2* | 2.7** | 18.3* | 17.9* |
| 1023A | 30.2 | 40.9 | 11.8* | 3.3** | 19.2* | 18.9* |
| 1023C | 34.5* | 41.7 | 12.1* | 3.6** | 18.4* | 18.0* |
| 1023G | 29.9 | 41.5 | 11.9* | 3.5** | 19.2* | 19.1* |
| 1023J | 32.1 | 42.0 | 14.4* | 3.5** | 25.7* | 25.7* |
| NBt Control | 24.2 | 45.4 | 2.3 | 49.0 | 1.7 | 1.4 |
| SE | 5.7 | 1.6 | 1.5 | 3.3 | 3.9 | 3.5 |
| Pr > F | 0.0783 | 0.3703 | 0.0086 | <0.0001 | 0.0104 | 0.0074 |

Contrasts statistics: *$P \leq 0.05$; **$P \leq 0.001$.

although numerically higher, were not significantly different from the non-Bt control plants.

For Ghana in 2016, non-Bt plants were significantly taller (cm) than non-Bt plants ($F_{1, 3} = 18.4$, $P = 0.0232$; non-Bt = 71.5, Bt = 53.3, SE = 3.4); and Bt plants produced first flowers about 2 d sooner than non-Bt plants ($F_{1, 3} = 13.4$, $P = 0.0354$; non-Bt = 44.0, Bt = 42.3, SE = 0.9). Furthermore, Bt-cowpea plants compared with the non-Bt-cowpea plants produced significantly more pods per plant ($F_{1, 3} = 745.4$, $P < 0.0001$; non-Bt = 1.8, Bt = 21.6, SE = 0.6), had lower percentage of pod damage ($F_{1, 3} = 255.6$, $P = 0.0005$; non-Bt = 71.8, Bt = 0.23, SE = 3.2), had higher total seed weight (g) per plant ($F_{1, 3} = 774.9$, $P < 0.0001$; non-Bt = 1.1, Bt = 23.1, SE = 0.6), and higher healthy seed weight (g) per plant ($F_{1, 2.99} = 709.2$, $P < 0.0001$; non-Bt = 0.38, Bt = 23.1, SE = 0.6).

## Discussion

High *M. vitrata* pressure provided by manual artificial infestations of all plants in the CFTs at the three locations clearly highlighted the efficacy of the Bt-cowpea events and introgressed line PBR Cowpea. All the cowpea Bt events consistently had less damage from *M. vitrata* compared to the non-Bt near-isogenic control (IT86D-1010) (Tables 1 and 2). *Maruca vitrata* infests cowpea flower buds, flowers, and pods. Without spraying insecticides, growers can have devastating losses (Singh et al. 1990). The effective protection against *M. vitrata* conferred by the *cry1Ab* gene is particularly evident in the 2012 study where all Bt-cowpea events produced significantly higher number of pods per plant compared with the non-Bt isoline resulting in significantly higher seed yield in Nigeria and Burkina Faso. High expression of Cry1Ab protein in the leaf, flower and pod tissues contributes to this control (Ba et al. 2018). This high level of control is similar to that found in Cry1Ab sweet corn, which was 99–100% in a Minnesota study (Burkness et al. 2001). These similar results were anticipated because *O. nubilalis* and *M. vitrata* are related, both in the family Crambidae.

The *cry1Ab* gene was transferred successfully to a farmer-preferred cowpea variety SAMPEA 10 to produce PBR Cowpea through conventional breeding. This Bt-cowpea, was highly effective at controlling pod damage by *M. vitrata* in the three country study (Table 3). The near-complete control of bean pod borer damage by this line resulted in significantly higher pod production in comparison to its non-Bt near isoline. The latter line did not produce any healthy seeds in Burkina Faso, a phenomenon that occurs when growers do not spray their crops with insecticides (Singh et al. 1990). Typically, growers have been spraying five to eight times in a cropping season (Jackai and Adalla 1997). Interestingly, total and healthy seed weights per plant were not significantly different in the 2015 study. This probably is due to the results from Ghana. In this

**Table 3.** Nigeria, Burkina Faso and Ghana 2015: Evaluation of Pod Borer Resistant cowpea Bt line (PBRC), and their near-isogenic non-Bt control (PBC) with means for plant height, days to first flowering, total pods per plant, percent pods with *M. vitrata* pod damage, and total and healthy seed weights per plant; and ANOVA statistics with SE

| Bt Events/ Non-Bt Control | Plant height (cm) | Days to first flowering | Total pods/ plant | *M. vitrata* pod damage (%) | Total seed weight/plant (g) | Healthy seed weight/plant (g) |
|---|---|---|---|---|---|---|
| Bt (PBRC) | 44.5 | 38.9 | 18.0 | 0.2 | 23.2 | 22.5 |
| Non-Bt (PBC) | 48.1 | 40.8 | 6.9 | 43.7 | 10.1 | 7.7 |
| SE | 18.8 | 2.5 | 2.5 | 1.1 | 4.2 | 4.3 |
| Pr > F | 0.8351 | 0.4190 | 0.0344 | <0.0001 | 0.0904 | <0.0722 |

case, the trial was planted late and plant development extended into the dry season, which required irrigation for the plants to survive. The irrigation may have helped the non-Bt plants to compensate for pod damage and resulted in similar total seed weight per plant (non-Bt = 13.8, Bt = 14.0, SE = 1.5). On the other hand, when this trial was planted at a normal time in 2016 and irrigation was not required, the non-Bt plants, as expected, produced significantly lower total seed weight per plant (non-Bt = 1.1, Bt = 23.1, SE = 0.6).

Plant development measures (height and days to first flowering) in most cases were not significantly different. Although, in 2012 (Table 2), a couple of the events were significantly taller than the control, but in 2016 the reverse occurred. In the 2016 study, Bt-cowpea first flowering occurred about 2 d before the non-Bt-cowpea. Such site-to-site variation is not unexpected due to several possible agronomic factors including water quantity and growing period.

After introduction, Bt-cowpea is expected to reduce the number of insecticidal sprays significantly for growers in the Guinea Savanna and Sudan Savanna agro-ecological zones in sub-Saharan Africa due to its high efficacy in controlling bean pod borer, *M. vitrata*. However, besides *M. vitrata*, cowpea production is impacted by a wide range of additional pests, which are not affected by Cry1Ab protein, including aphids, thrips, and pod sucking bugs, and they too often reduce cowpea yields (Singh et al. 1990). Thus, while Bt-cowpea will play an effective role in controlling the critical key pest *M. vitrata*, it will be integrated into a more comprehensive integrated pest management program in order to attain successful management of the entire pest complex. Furthermore, an insect resistance management (IRM) strategy will be implemented to protect Bt-cowpea from *M. vitrata* evolving resistance (Huesing et al. 2011), especially in areas where *M. vitrata* is endemic (Onstad et al. 2012). The IRM plan is based on the high-dose refuge strategy (Roush 1997, Gould 1998). Initially, the IRM plan for Bt-cowpea will require 50% non-Bt refuge fields within 400 m of Bt-cowpea. Growers will be encouraged to maintain land races as a component of this non-Bt refuge, which will help maintain genetic diversity. In many areas where Bt-cowpea will be released, natural refuges (Agunbiade et al. 2014) are expected to contribute *M. vitrata* susceptible to Cry1Ab protein. Additionally, Bt-cowpea releases will be limited until a two-toxin pyramid is available by restricting the number of varieties in which the trait is introgressed and by monitoring Bt-cowpea adoption levels. Pyramids more durable to insect resistance have been achieved successfully in other crops (Zhao et al. 2003, Tabashnik and Carrière 2017, Naranjo et al. 2020).

Bt crops such as maize and cotton have been effective in controlling lepidopteran insect pests for many years and their safety have led to their wide use (James 2014, Naranjo et al. 2020). Bt-cowpea is expected to control bean pod borer effectively when released to growers.

## Conclusions

Effective control of bean pod borer, *M. vitrata*, with the *cry1Ab* Bt gene in cowpea has been decisively demonstrated in these CFTs conducted in Guinea and Sudan Savanna agro-ecological zones in West Africa where cowpea is commonly grown. Bt-cowpea controls both pod and seed damage effectively, resulting in significant seed yield protection. These efficacy trials are an important component of the regulatory safety package and were developed and evaluated by in-country scientists. This information serves to assure regulators and growers in West Africa that Bt-cowpea is effective in safely controlling *M. vitrata*. Since cowpea commonly is infested with many other insects besides *M. vitrata*, an integrated pest management strategy that includes both Bt-cowpea biological control and/or insecticide sprays to control other cowpea pests will be developed to enhance cowpea production. An insect resistance management strategy also will be implemented to protect Bt-cowpea from *M. vitrata* evolving resistance. In January 2019, a permit for the commercial release of PBR Cowpea was granted by the Nigerian National Biosafety Management Agency (NBMA). In December 2019, the National Variety Release Committee of Nigeria approved Bt-cowpea for farmer use in Nigeria.

## Acknowledgments

The authors are thankful to the African Agricultural Technology Foundation (AATF) for coordinating and providing funding for the pod-borer resistant cowpea (PBRC) project. The authors also are thankful to the Australian Commonwealth Scientific and Industrial Research Organisation (CSIRO, R-04676-01), the United States Agency for International Development (USAID, AID-OAA-A-14-00035), the Institute for Agricultural Research (IAR), Ahmadu Bello University (ABU) and National Biotechnology Development Agency (NABDA) in Nigeria, Institut de l'Environnement et de Recherches Agricoles (INERA) in Burkina Faso, and the Savanna Agricultural Research Institute (SARI) in Ghana for providing funds and support for the PBRC project. Monsanto (now Bayer) donated the Bt gene royalty-free for use in the project; Program for Biosafety Systems (PBS) provided regulatory advice; and Kirkhouse Trust provided the marker assisted selection technology. We are also grateful to Purdue University, Rockefeller Foundation, the Network for the Genetic Improvement of Cowpea for Africa (NGICA), Danforth Foundation, and International Institute of Tropical Agriculture (IITA). We are thankful for the contributions to this project that were made by Jeremy T. Ouedraogo, and Eugene Terry. We are especially grateful to Larry Murdock for his initiation of many collaborations that led to this success. Finally, we are grateful to Hamza Adamu at IAR, Herve Bama at INERA, and Ahmed Seidu at SARI, for their field technical assistance. The authors have not declared any conflict of interests.

## References Cited


**Abudulai, M., F. Kusi, S. S. Seini, A. Seidu, J. A. Nboyine, and A. Larbi. 2017.** Effects of planting date, cultivar and insecticide spray application for the management of insect pests of cowpea in northern Ghana. Crop Prot. 100: 168–176.

**Agunbiade, T. A., B. S. Coates, B. Datinon, R. Djouaka, W. Sun, M. Tamò, and B. R. Pittendrigh. 2014.** Genetic differentiation among *Maruca vitrata* F. (Lepidoptera: Crambidae) populations on cultivated cowpea and wild host plants: implications for insect resistance management and biological control strategies. PLoS One. 9: e92072.

**Ba, M. N., J. E. Huesing, M. Tamò, T. J. Higgins, B. R. Pittendrigh, and L. L. Murdock. 2018.** An assessment of the risk of Bt-cowpea to non-target organisms in West Africa. J. Pest Sci. 91: 1165–1179.

**Ba, M. N., J. E. Huesing, C. L. Dabiré-Binso, M. Tamò, B. R. Pittendrigh, and L. L. Murdock. 2019.** The legume pod borer, *Maruca vitrata* Fabricius (Lepidoptera: Crambidae), an important insect pest of cowpea: a review emphasizing West Africa. Int. J. Trop. Insect Sci. 39: 93–106.

**Bassey, F. I., K. H. McWatters, C. A. Edem, and C. M. Iwegbue. 2013.** Formulation and nutritional evaluation of weaning food processed from cooking banana, supplemented with cowpea and peanut. Food Sci. Nutr. 1: 384–391.

**Boukar, O., C. A., Fatokun, P. A. Roberts, M. Abberton, B. L. Huynh, T. J. Close, S. Kyei-Boahen, T. J. Higgins, and J. D. Ehlers. 2015.** Cowpea, pp. 219–250. *In* A. M. De Ron (ed.), Grain Legumes (Handbook of Plant Breeding 10). Springer, New York, NY.

**Bressani, R. 1985.** Nutritive value of cowpea, pp. 353–356. *In* S. R. Singh, K. O. Rachie (eds.), Cowpea Research, Production and Utilization. John Wiley and Sons, New York, NY.

**Brookes, G., and P. Barfoot. 2018.** Environmental impacts of genetically modified (GM) crop use 1996-2016: impacts on pesticide use and carbon emissions. GM Crops Food. 9: 109–139.

**Burkness, E. C., W. D. Hutchison, P. C. Bolin, D. W. Bartels, D. F. Warnock, and D. W. Davis. 2001.** Field efficacy of sweet corn hybrids expressing a *Bacillus thuringiensis* toxin for management of *Ostrinia nubilalis* (Lepidoptera: Crambidae) and *Helicoverpa zea* (Lepidoptera: Noctuidae). J. Econ. Entomol. 94: 197–203.

**Dugje, I. Y., L. O. Omoigui, F. Ekeleme, A. Y. Kamara, and H. Ajeigbe. 2009.** Farmers' Guide to Cowpea Production in West Africa, 20 pp. International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

**Ekesi S. 1999.** Insecticide resistance in field populations of the legume pod-borer, *Maruca vitrata* Fabricius (Lepidoptera: Pyralidae), on cowpea, *Vigna unguiculata* (L.), Walp in Nigeria. Int. J. Pest Manage. 45: 57–59.

**Gould, F. 1998.** Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. Annu. Rev. Entomol. 43: 701–726.

**Halsey, M. E. 2006.** Integrated confinement system for genetically engineered plants. IFPRI Program for Biosafety Systems (PBS) Available from https://www.ifpri.org/publication/integrated-confinement-system-genetically-engineered-plants.

**Higgins, T. J. V., S. Gollasch, L. Molvig, A. Moore, C. Popelka, J. Armstrong, R. Mahon, J. Ehlers, J. Huesing, V. Margam, *et al.* 2012.** Insect-protected cowpeas using gene technology, pp. 131–137. *In* O. Boukar, O. Coulibaly, C. Fatokun, K. Lopez, and M. Tamo (eds.), Proceedings of the fifth world cowpea conference on improving livelihoods in the cowpea value chain through advancement in science. 27 September – 1 October 2010, Saly, Senegal. International Institute of Tropical Agriculture, Ibadan, Nigeria.

**Huesing, J. E., J. Romeis, N. C. Ellstrand, A. Raybould, R. L. Hellmich, J. D. Wolt, J. D. Ehlers, L. C. Dabiré, C. A. Fatokun, K. E. Hokanson, *et al.* 2011.** Regulatory considerations surrounding the deployment of Bt-expressing cowpea in Africa: report of the deliberations of an expert panel. GM Crops. 2: 211–224.

**Jackai, L. E. N., and C. B. Adalla. 1997.** Pest management practices in cowpea: a review, pp. 240–258. *In* B. B. Singh, D. R. Mohan Raj, K. E. Dashiell, and L. E. N. Jackai (eds.), Advances in cowpea research. Co- publication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria.

**Jackai, L. E. N., and R. A. Daoust. 1986.** Insect pests of cowpeas. Annu. Rev. Entomol. 31: 95–119.

**Jackai, L. E. N. and J. R. Raulston. 1988.** Rearing the legume pod borer, *Maruca testulalis* Geyer (Lepidoptera: Pyralidae) on artificial diet. Int. J. Pest Manage. 34: 168–172.

**James, C. 2014.** Global status of commercialized biotech/GM crops. ISAAA Brief No 49, Ithaca, NY Available from https://isaaa.org/resources/publications/briefs/49/default.asp.

**Kamara, Y., D. Chikoye, L. O. Omoigui, and I. Y. Dugje. 2007.** Influence of insecticide spraying regimes and cultivar on insect pests and yield of cowpea in the dry savannas of north-eastern Nigeria. J. Food Agric. & Environ. 5: 154–158.

**Kamara, A. Y., A. I. Tofa, S. Kyei-Boahen, R. Solomon, H. A. Ajeigbe, and N. Kamai. 2016.** Effects of plant density on the performance of cowpea in Nigerian Savannas. Expl. Agric. 54: 120–132.

**Klümper, W., and M. Qaim. 2014.** A meta-analysis of the impacts of genetically modified crops. PLoS One 9: e111629.

**Mohammed, B. S., M. F. Ishiyaku, U. S. Abdullahi, and M. D. Katung. 2014.** Response of transgenic Bt cowpea lines and their hybrids under field conditions. J. Plant Breed. Crop Sci. 6: 91–96.

**Murdock L. M., O. Coulibaly, T. J. V. Higgins, J. E. Huesing, M. F. Ishiyaku, and I. Sithole-Niang. 2008.** Cowpea: legume grains and forages, pp. 23–56. *In* C. Kole and T. C. Hall (eds.), A compendium of transgenic crop plants. Blackwell Publishing, Oxford, UK.

**Naranjo, S. E., R. L. Hellmich, J. Romeis, A. M. Shelton, and A. M. Vélez. 2020.** The role and use of genetically engineered insect-resistant crops in IPM systems, pp. 283–340. *In* M. Kogan and E. Heinrichs (eds.), Integrated management of insect pests: current and future developments. Burleigh Dodds Science Publishing, Cambridge, UK.

**National Biosafety Management Agency (NBMA), Nigeria. 2019.** Decision Document for a permit for the Commercial release of Pod Borer - Resistant Cowpea (PBR – Cowpea)-event AAT709A, genetically modified for lepidopteran insect pest (*Maruca vitrata*) resistance, issued to Institute for Agricultural Research (IAR), Zaria. Available from https://nbma.gov.ng/wp-content/uploads/2017/11/Decision-Document-for-Commercial-Release-of-PBR-Cowpea-2019-final.pdf

**Onstad, D. W., J. Kang, N. M. Ba, M. Tamò, L. Jackai, C. Dabire, and B. R. Pittendrigh. 2012.** Modeling evolution of resistance by *Maruca vitrata* (Lepidoptera: Crambidae) to transgenic insecticidal cowpea in Africa. Environ. Entomol. 41: 1255–1267.

**Popelka, J. C., S. Gollasch, A. Moore, L. Molvig, and T. J. Higgins. 2006.** Genetic transformation of cowpea (*Vigna unguiculata* L.) and stable transmission of the transgenes to progeny. Plant Cell Rep. 25: 304–312.

**Roush, R. T. 1997.** Bt-transgenic crops: just another pretty insecticide or a chance for a new start in resistance management? Pestic. Sci. 51: 328–334.

**SAS®. Version 9.4. 2015.** SAS Institute, Inc., Cary, NC.

**Schmidt, M., and W. Parrott. 2001.** Quantitative detection of transgenes in soybean [*Glycine max* (L.) Merrill] and peanut (*Arachis hypogaea* L.) by real-time polymerase chain reaction. Plant Cell Rep. 20: 422–428.

**Shelton, A. M., M. J. Hossain, V. Paranjape, A. K. Azad, M. L. Rahman, A. S. M. M. R. Khan, M. Z. H. Prodhan, M. A. Rashid, R. Majumder, M. A. Hossain, *et al.* 2018.** Bt eggplant project in Bangladesh: history, present status, and future direction. Front. Bioeng. Biotechnol. 6: 106.

**Singh, S. R., and H. F. van Emden. 1979.** Insect pests of grain legumes. Annu. Rev. Entomol. 24: 255–278.

**Singh, S. R., L. E. N. Jackai, J. H. R. Dos Santos, and C. B. Adalla. 1990.** Insect pests of cowpea, pp. 43–89. *In* S. R. Singh (ed.), Insect pests of food legumes. John Wiley & Sons Ltd., New York, NY.

**Srinivasan, R. 2008.** Susceptibility of legume pod borer (LPB), *Maruca vitrata* to delta-endotoxins of *Bacillus thuringiensis* (Bt) in Taiwan. J. Invertebr. Pathol. 97: 79–81.

**Tabashnik, B. E., and Y. Carrière. 2017.** Surge in insect resistance to transgenic crops and prospects for sustainability. Nat. Biotechnol. 35: 926–935.

**Taylor, T. A. 1967.** The bionomics of *Maruca testululis* Gey. (Lepidoptera: Pyralidae), a major pest of cowpeas in Nigeria. J. West Afr. Sci. Assoc. 12: 111–129.

**Zhao, J. Z., J. Cao, Y. Li, H. L. Collins, R. T. Roush, E. D. Earle, and A. M. Shelton. 2003.** Transgenic plants expressing two *Bacillus thuringiensis* toxins delay insect resistance evolution. Nat. Biotechnol. 21: 1493–1497.