

Article

Field Performance of a Genetically Modified Cowpea (*Vigna unguiculata*) Expressing the Cry1Ab Insecticidal Protein Against the Legume Pod Borer *Maruca vitrata*

Jerry A. Nboyine ^{1,*}, Gloria A. Adazebra ¹, Emmanuel Y. Owusu ¹, Philip Agrengsore ¹, Ahmed Seidu ¹, Salim Lamini ¹, Mukhtaru Zakaria ¹, James Y. Kwabena ¹, Haruna K. Ali ¹, Ijeoma Akaogu ², Francis N. Onyekachi ², Jean B. Tignegre ², Prince M. Etwire ¹ , Donald J. MacKenzie ³ , Jose M. Barrero ⁴  and Thomas J. V. Higgins ⁴ 

- ¹ Council for Scientific and Industrial Research, Savanna Agricultural Research Institute, Tamale P.O. Box 52, Ghana; gloriaadazebra@yahoo.com (G.A.A.); owusuemmagh@yahoo.com (E.Y.O.); philip.agrengsore@yahoo.com (P.A.); seiduahmedk730@yahoo.com (A.S.); salim.lamini@gmail.com (S.L.); balamutar67@gmail.com (M.Z.); kwabenajamesyaw1@gmail.com (J.Y.K.); harunamisbau@gmail.com (H.K.A.); etwiremaxwellprince@gmail.com (P.M.E.)
 - ² African Agricultural Technology Foundation (AATF), 162 Independence Avenue, CBD, Abuja 900211, Nigeria; i.akaogu@aاتف-africa.org (I.A.); f.onyekachi@aاتف-africa.org (F.N.O.); j.tignegre@aاتف-africa.org (J.B.T.)
 - ³ International Institute for Crop Improvement, Donald Danforth Plant Science Centre, St. Louis, MO 63132, USA; dmackenzie@danforthcenter.org
 - ⁴ Commonwealth Scientific and Industrial Research Organization, Agriculture and Food, Canberra, ACT 2601, Australia; jose.barrero@csiro.au (J.M.B.)
- * Correspondence: nboyinejerry@gmail.com

Abstract: Cowpea (*Vigna unguiculata*) is a vital crop in sub-Saharan Africa, but the legume pod borer (LPB), *Maruca vitrata*, can cause over 80% yield losses. Natural resistance to this lepidopteran pest is absent in cowpea germplasm, and insecticides are ineffective due to the pest's cryptic behavior. To address this, a genetically modified (GM) cowpea expressing the cry1Ab protein from *Bacillus thuringiensis* (Bt) was developed, providing complete LPB resistance. This Bt cowpea, commercialized as Sampea 20-T in Nigeria, was recently approved in Ghana as Songotra T. To evaluate its performance and the financial returns of its cultivation, field trials were conducted across multiple locations in northern Ghana to compare it to the non-transgenic Songotra control and two commercial cultivars, Kirkhouse-Benga and Wang-Kae. Songotra T exhibited protection against LPB infestations and damage, achieving a grain yield of 2534 kg/ha compared to 1414–1757 kg/ha for the other entries. As expected, non-LPB pest infestations and damage were similar across all entries. Economic analysis revealed that Songotra T had the highest return on investment (464%), outperforming the other tested cultivars (214%). These results demonstrate the potential of GM crops to enhance yields and profitability for resource-poor farmers, underscoring the value of biotechnology for addressing critical agricultural challenges.

Keywords: *cry1Ab* gene; genetic modification; grain yield; pest damage; return on investment



Citation: Nboyine, J.A.; Adazebra, G.A.; Owusu, E.Y.; Agrengsore, P.; Seidu, A.; Lamini, S.; Zakaria, M.; Kwabena, J.Y.; Ali, H.K.; Akaogu, I.; et al. Field Performance of a Genetically Modified Cowpea (*Vigna unguiculata*) Expressing the Cry1Ab Insecticidal Protein Against the Legume Pod Borer *Maruca vitrata*. *Agronomy* **2024**, *14*, 3055. <https://doi.org/10.3390/agronomy14123055>

Academic Editor: Maolin Hou

Received: 25 November 2024

Revised: 16 December 2024

Accepted: 18 December 2024

Published: 21 December 2024



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1. Introduction

Cowpea (*Vigna unguiculata* (Linn.) Walp.) is a major food grain legume that is widely consumed in sub-Saharan Africa (SSA) [1]. The edible parts are the grains, the green pods, and the leaves, which are rich in proteins (23–30%), essential vitamins (e.g., thiamine, folic acid, and niacin), iron, and dietary fibre [2]. Cowpea cultivation is estimated to be profitable in SSA. A study by [3] reported a net farm income of USD 810/ha. Similarly, [4] reported a net positive profit for cowpea production when improved varieties were combined with effective pest management strategies. In spite of the importance of this crop in SSA and beyond, most consuming countries are unable to produce sufficient quantities to meet domestic demands because of reduced grain yields in farmers' fields (200–400 kg/ha) [5].

These low yields are mainly caused by insect pests that attack the crop from the vegetative to reproductive stages [6,7].

The range of economically important pests that attack cowpea in the field are broadly grouped into vegetative, flowering, and podding stage pests. The most damaging ones are those that occur at the flowering and podding stages [8–10]. Major vegetative stage pests of cowpea are the cowpea aphid (*Aphis craccivora* Koch, Hemiptera: Aphididae), foliage beetles (*Ootheca* spp., Coleoptera: Chrysomelidae; *Medythia* spp., Coleoptera: Chrysomelidae), leaf miners (*Liriomyza sativa* Blanch., Diptera: Agromyzidae), whiteflies (*Bemisia tabaci* Genn., Hemiptera: Aleyrodidae), and leafhoppers (*Empoasca* sp., Hemiptera: Cicadellidae) [10–12]. At the flowering stage, thrips (*Megalurothrips sjostedti* Try., Thysanoptera: Thripidae) and the legume pod borer (LPB) (*Maruca vitrata* F., Lepidoptera: Crambidae), are economically important, while the LPB and the pod-sucking bugs (PSBs) (*Clavigralla tomentosicollis* Stal, Hemiptera: Coreidae; *Anoplocnemis curvipes* (F.), Hemiptera: Coreidae; *Riptortus dentipes* (F.), Hemiptera: Alydidae; *Nezara viridula* L., Hemiptera: Pentatomidae; *Thyanta custator* (F.), Hemiptera: Pentatomidae; and *Aspavia armigera* (L.), Hemiptera: Pentatomidae are the most important at the pod formation and filling stages [6,7,12].

In West Africa, the cowpea yield deficits, despite insecticide use, are in significant proportion attributable to infestation and damage by the LPB, which occurs at the flowering and podding stages [13–15]. This is because the LPB, like other pests in the Crambidae family, feeds internally; thus, it is protected from insecticide solutions sprayed externally [13]. Apart from insecticide use, natural regulation of the LPB population in cowpea fields is thought to occur through the activities of parasitoids, such as *Apanteles taragamae* (Hymenoptera: Braconidae), *Bassus asper* (Hymenoptera: Braconidae), *Dolichogenidea* sp. (Hymenoptera: Braconidae), *Trichomma* sp. (Hymenoptera: Ichneumonidae), *Triclistus* sp. (Hymenoptera: Ichneumonidae), and *Plectochorus* sp. (Hymenoptera: Ichneumonidae), and predatory staphylinids [14,16–18]. However, over-reliance on synthetic insecticides for the management of LPBs and other economically important insect pests of cowpea negatively impacts these beneficial arthropods, thereby decreasing their contribution to LPB population reductions [19].

The development and deployment of improved cowpea varieties resistant to LPBs would be an environmentally friendly measure to mitigate LPB damage, reduce insecticide use, and increase yields in a sustainable manner [20]. However, intensive screening of global germplasm for sources of resistance to LPBs found none in cultivated cowpeas, except in a few wild accessions. The levels of resistance in these wild cowpeas were too low to have any breeding value. Also, reproductive barriers between the wild and cultivated cowpeas affected efforts at transferring resistance to domesticated cowpea [15,20,21].

To overcome this lack of host plant resistance, cowpea was genetically modified to produce transgenic event AAT-7Ø9AA-4 using *Agrobacterium tumefaciens* [(Smith & Townsend 1907) Conn 1942 (Hyphomicrobiales: Rhizobiaceae)]-mediated transformation of embryogenic seed explants of the cultivar IT86D-1010 with plasmid pMB4. This resulted in the introduction of the *cry1Ab* gene from *Bacillus thuringiensis* subsp. *kurstaki* strain HD-1 and the neomycin phosphotransferase II encoding gene (*nptII*) from the Tn5 transposon of *Escherichia coli* strain K12 as a selectable marker [22]. The introduction of this gene leads to the expression of Cry1Ab insecticidal protein. The primary action of the Cry1Ab toxin is to lyse midgut epithelial cells of the pest by forming pores in the apical microvilli membrane of the cells, leading to death of the insect [23,24]. This cowpea event and its progeny (derived by introgressing the *cry1Ab* gene from AAT-7Ø9AA-4 into the commercial cowpea variety called Songotra (IT97K-499-35)) were reported to provide full protection against LPB damage when they were tested in screenhouses under confined field trials [25]. But, the performance of this transgenic cowpea (hereafter referred to as Songora T) under typical field conditions, which are characterized by multiple cowpea pests' complex infestations, has not been studied yet. Hence, the objective of this study was to assess the performance of the Songotra T expressing the Cry1Ab insecticidal protein and the financial returns of cultivating this transgenic cowpea compared to conventional ones.

2. Materials and Methods

2.1. Study Sites

Field experiments were conducted under rain-fed conditions in four of the major cowpea-producing regions in Ghana during the 2023 growing season. These locations are major cowpea testing sites in northern Ghana [26]. The locations were Akukayili (latitude: $09^{\circ}23'32''$; longitude: $001^{\circ}00'14''$; 199 a.s.l), Settlement (latitude: $09^{\circ}03'22''$; longitude: $001^{\circ}45'53''$; 171 a.s.l), Kpasenkpe (latitude: $10^{\circ}28'20''$; longitude: $001^{\circ}01'32''$; 135 a.s.l), and Chinchang (latitude: $10^{\circ}52'06''$; longitude: $001^{\circ}54'51''$; 305 a.s.l) (Figure 1). These trial sites are located in the Guinea Savannah zone and are characterized by unimodal rainfall patterns, which commence in May and end in October of each year. The Köppen–Geiger classification system categorizes the climate in these locations as a Tropical Savannah climate with non-seasonal or dry-winter characteristics (Aw) [27]. Annual rainfall in these study sites ranges between 900 and 1200 mm, while temperatures range between 20 and 35 °C during the growing season [28].



Figure 1. Map of northern Ghana showing the spatial distribution of sites for field testing of the performance of test entries. Note: Akukayili is in the Tolon District of the Northern Region; Chinchang is in the Sissala East District of the Upper West Region; Kpasenkpe is in the West Mamprusi Municipality of the North East Region; and Settlement is in the West Gonja Municipality of the Savannah Region.

2.2. Description of Test Entries

The plant materials tested were Songotra T, Songotra, Wang-Kae, and Kirkhouse-Benga. Brief descriptions of the test materials are provided below.

1. Songotra T—this was developed by introgressing the *cry1Ab* gene from the *Bacillus thuringiensis* subsp. *kurstaki* strain HD-1 into a commercially released cowpea variety called Songotra (IT97K-499-35). The cowpea event AAT-709AA-4, produced through *Agrobacterium-tumefaciens*-mediated transformation of the genotype IT86D-1010, was used as a donor. The Songotra T has an erect growth habit with a determinate growth pattern and a non-twining tendency. The color of the flowers is white with a yellow throat. The seed size and color are large and creamy white, respectively. It is highly resistant to the parasitic plant *Striga gesnerioides* (Willd.) Vatke, and it matures early (62–65 days). It is resistant to the LPB and charcoal rot disease caused by *Macrophomina phaseolina* (Tassi). It has a yield potential of 2.5 t/ha. This variety, referred to as Songotra T in Ghana, has the commercial name Sampea 20-T in Nigeria. Thus, Songotra T and Sampea 20-T refer to the same genotype, which has different commercial names in two different countries.
2. Songotra—it was released by the CSIR–Savanna Agricultural Research Institute (CSIR–SARI) in 2008 for cultivation in the Sahel, Sudan, and Guinea Savannah zones of Ghana. The pure line is IT97K-499-35. It generally exhibits all of the characteristics of Songotra T, including a yield potential of 2.5 t/ha, except resistance to LPBs and charcoal rot disease [29].
3. Wang-Kae—it was released by CSIR–SARI in 2016 for cultivation in the Guinea and Sudan Savannah zones as well as the Transitional, Coastal Savannah, and Forest zones. It is a cross between IT99K-573-1-1 and SARC 1-57-2. It has an erect growth habit with few vines (i.e., semi-spreading). The growth pattern is determinate, and the color of the flowers is white. Most pods are above the canopy. It has a yield potential of 2.4 t/h, and it matures early (i.e., 65 days after planting). It is highly resistant to *Aphis craccivora* and *S. gesnerioides* but moderately tolerant to charcoal rot disease [29].
4. Kirkhouse-Benga—it was released in 2016 by CSIR–SARI for cultivation in the Guinea and Sudan Savannah, Transitional, Coastal Savannah, and Forest zones of Ghana. It is a cross between IT99K-573-2-1 and SARC 1-57-2. It has an erect growth habit without vines. The color of its flowers is white with purple marks. In terms of pod positioning, the majority are slightly above the canopy. The potential yield of this variety is 2.4 t/ha, and it matures between 60 and 65 days. This variety is also highly resistant to *A. craccivora* and *S. gesnerioides* [29].

Note: although the yield potential of conventional cowpeas (irrespective of the variety) is generally higher than 2 t/ha, the maximum actual (or common) yields recorded in farmers' fields are 1.5 t/ha [30]. Yield potential figures are based on the genetic potential of the plant material/variety in the absence of or under minimal biotic and abiotic stresses as well as under the adoption of optimum agronomic practices.

2.3. Field Layout and Trial Management

All trial fields were tractor ploughed followed by harrowing to obtain a fine tilth of the soil. The test entries (Songotra T, Songotra, Wang-Kae, and Kirkhouse-Benga) were then arranged in a randomized complete block design with four replications. Plot sizes comprised 4 rows of cowpea that were 5 m long. Two seeds were sown per hill. Spacing of 60 cm between rows and 20 cm between plants was used at all locations. Plots were separated by a 2 m wide alley, while blocks were 3 m apart. Insect pests were controlled by applying K-Optimal (Lambda-Cyhalothrin 15 g/L + Acetamiprid 20 g/L) (Kumark Company, Kumasi, Ghana), at a rate of 0.5 L/ha. Insecticide sprays were applied twice, once at 50% flowering and once at 50% podding, using a CP-15 Knapsack sprayer (Agri-gem Ltd., Saxilby, UK).

The pre-emergence herbicide, Alligator 400 EC (pendimethalin, 400 g a.i. L⁻¹) (Kumark Company, Kumasi, Ghana), was applied at 2 L/ha immediately after planting to suppress weed (grasses) growth. This was followed by hand-weeding of the broad-leaved weeds at three weeks after sowing. In this study, fertilizers and fungicides were not applied.

2.4. Data Collection

Data were collected on the abundance of insect pests, natural predators, and pod and grain damage, as well as the grain yield. Briefly, the data were collected as described below.

1. Vegetative stage pests—whiteflies (*B. tabaci*) and leafhoppers (*Empoasca* sp.) were sampled using a sweep net at 21 days after planting. Each plot was swept twice, and the content of the sweep net was carefully transferred into labeled vials containing 70% ethanol. The vials were transported to the Entomology laboratory at the CSIR–Savanna Agricultural Research Institute (CSIR–SARI), and their contents were sorted for identification using a dichotomous key [31]. The numbers of whiteflies and leafhoppers in each vial were then counted. The mean numbers of these vegetative stage pests per treatment plot were then computed before proceeding with data analysis.
2. Flowering stage pests—at 90% flowering, thrips (*M. sjostedti*) and legume pod borers (LPBs) (*M. vitrata*) were sampled by randomly collecting 20 flowers from each plot into a vial containing 70% ethanol. These were transported to the laboratory and then sorted followed by identification using a dichotomous key [31] before counting. The numbers of thrips and LPBs were recorded.
3. Podding stage pest—the pod-sucking bug (PSB) population was estimated by counting nymphs and adults of the different species of sucking bugs in a 1 m stretch of the two middle rows in each plot [31]. These data were collected twice (i.e., at 50% and 90% of podding), and the PSB population was estimated as an average of the total number of PSB/m.
4. Natural enemy assemblage—the number of predators on 10 randomly selected plants was assessed through visual counting at flowering and podding for each plot [31]. This was followed by computing the mean for each plot.
5. PSB damage to pods and grains—at 90% podding, pod damage was estimated by randomly selecting 10 plants in the two inner rows and counting the total number of PSB-damaged pods and computing the average pod damage per plant for each of these insects. Damage by PSBs was based on the characteristic shriveling of pods and, occasionally, the feeding punctures on pods. In contrast, grain damage was computed by randomly selecting 100 grains from each cowpea entry and sorting them into damaged and undamaged grains. Damaged grains were identified by their shriveled nature. The percentage of damaged grains was the same as the number of damaged grains [7,32].
6. LPB damage to pods and grains—this was estimated by counting the total number of LPB-damaged pods on 10 randomly selected plants in the two inner rows at 90% podding. The average damage per plant was then computed. Features used to distinguish LPB damage on pods from damage by other insects were the round, well-cut entry holes, which were plugged with webbed frass. Seed damage was assessed by randomly selecting 100 seeds from each plot and sorting them into damaged and undamaged. The main distinguishing feature of LPB-damaged grains was the bite by the larvae. The percentage of damaged grains was the same as the number of damaged grains [33].
7. Grain yield—this data was collected at maturity by harvesting the pods of plants in the two middle rows of each plot. These pods were handpicked. The harvested pods were sun-dried and threshed to separate the grains from the shells. Afterwards, the threshed grains were winnowed to remove debris. The grains from each plot were weighed and converted to kilograms per hectare for analysis.

2.5. Data Analysis

All data were subjected to a combined-site analysis of variance (ANOVA) using the Genstat® statistical package 12th edition after an initial assessment for normality and homogeneity of their variances. In these analyses, the explanatory variables were the locations and the cowpea entries. Prior to ANOVA, count data (which followed a Poisson distribution) were square root transformed ($\sqrt{x + 1}$) to ensure homogeneity of their variances. Means for variables that showed significant location effects were presented separately for each trial site and vice versa. Means were separated using Tukey's HSD post hoc tests at a 5% probability threshold.

The correlation analysis was conducted using the Genstat® statistical package 12th edition. The variables used for these analyses were flowering stage pests, PSBs, damage to grains and pods by PSBs and LPBs, and grain yield. To test whether the correlations were significant, a two-sided test was used to decide whether the value of the recorded correlation coefficient was close to zero or significantly different from zero.

2.6. Economic Analysis

This study utilized the return on investment method to assess the economic profitability of each cowpea variety under evaluation. Additionally, data on average cowpea yields/ha in Ghana and the cost of producing a ha of the crop under farmer management practices were included in the economic profitability analysis to enable direct comparison on returns on investment among the entries studied with national averages. Mathematically, the return on investment for the i^{th} cowpea variety (RoI_i) [34] can be expressed as

$$RoI_i = \left(\frac{TR_i - TC_i}{TC_i} \right) \times 100$$

where TR_i is the total revenue for the i^{th} cowpea variety and TC_i is the associated total costs. The total revenue was calculated by multiplying the cowpea yield by the unit price, whereas the total cost was determined by summing up all production expenses, ranging from land rental to winnowing and bagging.

3. Results

3.1. Pest Infestation

3.1.1. Vegetative Stage Pests

A combined-sites ANOVA for whiteflies showed a significant location effect ($F_{3,48} = 36.19$; $p < 0.001$) only. There was no significant cowpea entry effect at Akukayili ($F_{3,12} = 2.34$; $p = 0.125$), Chinchang ($F_{3,12} = 0.13$; $p = 0.939$), Settlement ($F_{3,12} = 0.59$; $p = 0.635$), or Kpasenkpe ($F_{3,12} = 0.30$; $p = 0.828$) for infestations by this pest. The mean whitefly infestation across test locations was 29.4 per sweep.

Again, there was a significant location effect ($F_{3,48} = 50.55$; $p < 0.001$) when data from the different test sites were combined and analyzed for leafhopper infestations. There were no significant differences among the cowpea entries for this pest at Akukayili ($F_{3,12} = 1.22$; $p = 0.344$), Chinchang ($F_{3,13} = 0.43$; $p = 0.737$), Settlement ($F_{3,12} = 0.85$; $p = 0.493$), or Kpasenkpe ($F_{3,12} = 2.33$; $p = 0.126$). Across test locations, the mean infestation by this insect was 30.5 per sweep.

3.1.2. Flowering and Podding Stage Pests

The number of thrips/20 flowers was significantly affected by the location ($F_{3,48} = 14.75$; $p < 0.001$) when data from the four sites were combined and analyzed. Infestations by thrips were not significantly different among the cowpea entries at Akukayili, ($F_{3,12} = 0.91$; $p = 0.466$), Kpasenkpe ($F_{3,12} = 0.32$; $p = 0.812$), or Settlement ($F_{3,12} = 0.99$; $p = 0.431$). The mean infestations at Akukayili, Kpasenkpe, and Settlement were 11.3, 19.9, and 3.4, respectively. In contrast, the number of thrips/20 flowers was significantly affected by the entries at Chinchang ($F_{3,12} = 3.93$; $p = 0.036$). At this location, infestation was highest in the Songotra

T and lowest in Kirkhouse Benga. There were no significant differences between Songotra T, Wang-Kae, and Songotra in terms of thrips infestations (Table 1).

The number of LPB/20 flowers was also significantly affected by the location ($F_{3,48} = 3.91$; $p = 0.014$). Flower infestations by this insect were significantly different among cowpea entries at Akukayili ($F_{3,12} = 24.64$; $p < 0.001$), Chinchang ($F_{3,12} = 9.15$; $p = 0.002$), Kpasenkpe ($F_{3,12} = 8.44$; $p = 0.003$), and Settlement ($F_{3,12} = 6.65$; $p = 0.007$). In general, LPB infestations were higher in the conventional cowpea entries and lowest in the Songotra T (Table 1).

The pod-sucking bugs (PSBs) present were *C. tomentosicollis*, *R. dentipes*, *A. curvipes*, *N. viridula*, *A. armigera*, and *T. custator*. Their mean numbers/m were not significantly affected by the test locations ($F_{3,48} = 2.22$; $p = 0.097$), cowpea entries ($F_{3,48} = 1.85$; $p = 0.151$), or location $\times$ cowpea entry ($F_{9,48} = 1.56$; $p = 0.156$) interaction effect. The mean of this variable was 21 PSBs/m across locations.

Table 1. Mean number of thrips and legume pod borers (LPBs) in flowers of different cowpea entries at four different locations in northern Ghana.

Location	Entry	No. of Thrips/20 Flowers	No. of LPB/20 Flowers
Akukayili	Kirkhouse Benga	14.3 (3.8 + 0.4) a	8.0 (3.0 + 0.2) a
	Songotra	11.0 (3.4 + 0.4) a	8.3 (3.0 + 0.2) a
	Songotra T	11.5 (3.5 + 0.4) a	0.0 (1.0 + 0.0) b
	Wang Kae	8.3 (3.0 + 0.3) a	5.5 (2.5 + 0.2) a
	<i>p</i> -value	0.466	<0.001
Chinchang	Kirkhouse Benga	6.5 (2.3 + 0.8) b	9.5 (3.2 + 0.2) a
	Songotra	12.8 (3.2 + 1.1) ab	6.3 (2.6 + 0.3) a
	Songotra T	38.3 (6.1 + 0.9) a	0.0 (1.0 + 0.0) b
	Wang Kae	28.0 (5.2 + 0.7) ab	6.5 (2.2 + 0.5) a
	<i>p</i> -value	0.036	0.002
Kpasenkpe	Kirkhouse Benga	23.3 (4.7 + 0.9) a	4.5 (2.3 + 0.3) a
	Songotra	19.8 (4.5 + 0.2) a	6.3 (2.6 + 0.3) a
	Songotra T	21.5 (4.7 + 0.5) a	0.0 (1.0 + 0.0) b
	Wang Kae	15.3 (4.0 + 0.3) a	6.5 (2.7 + 0.3) a
	<i>p</i> -value	0.812	0.003
Settlement	Kirkhouse Benga	3.3 (1.4 + 0.5) a	2.3 (1.8 + 0.6) ab
	Songotra	4.0 (1.5 + 1.2) a	2.5 (1.7 + 0.2) ab
	Songotra T	1.0 (1.4 + 0.5) a	0.0 (1.0 + 0.0) b
	Wang Kae	5.3 (2.6 + 0.9) a	6.0 (3.5 + 0.7) a
	<i>p</i> -value	0.431	0.007

Note: figures in brackets are square root [$\sqrt{(x + 1)}$] transformed means $\pm$ standard error of means; for each location, means followed by different letters are significantly different at 5% probability threshold.

3.1.3. Pod and Grain Damage

A combined-site analysis for pod damage by PSBs showed a significant location effect ($F_{3,48} = 17.99$; $p < 0.001$). Except for Settlement ($F_{3,12} = 21.75$; $p < 0.001$), PSB-damaged pods/plant were not significantly different among cowpea entries at Akukayili ($F_{3,12} = 0.92$; $p = 0.459$), Chinchang ($F_{3,12} = 0.15$; $p = 0.927$), or Kpasenkpe ($F_{3,12} = 1.02$; $p = 0.420$). The means of this variable at Akukayili, Chinchang, and Kpasenkpe were 2.0, 3.2, and 3.1, respectively. At Settlement, the mean PSB-damaged pods/plant was highest in Songotra T and lowest in Kirkhouse Benga. There were no significant differences between infestations in Kirkhouse Benga and Songotra or Wang-Kae (Table 2).

Similarly, seed damage by PSBs was significantly affected by the location ($F_{3,48} = 15.63$; $p < 0.001$). Except for Chinchang ($F_{3,12} = 4.18$; $p = 0.031$), the percentage of PSB-damaged seeds was not significantly different among entries at Akukayili ($F_{3,12} = 2.51$; $p = 0.108$), Kpasenkpe ($F_{3,12} = 3.38$; $p = 0.054$), or Settlement ($F_{3,12} = 0.16$; $p = 0.923$). The mean percentage of damage at Akukayili, Kpasenkpe, and Settlement was 15.1, 29.2, and 35.3, respectively. At Chinchang, this variable was highest in Songotra T and lowest in Kirkhouse

Benga. There was no significant difference between damage in the latter and Songotra or Wang-Kae (Table 2).

Damage to pods by LPBs was significantly affected by the location ($F_{3,48} = 24.06$; $p < 0.001$) when data from the four test locations were combined for analysis. This variable was significantly affected by the test entries at Akukayili ($F_{3,12} = 6.08$; $p = 0.009$), Chinchang ($F_{3,12} = 37.10$; $p < 0.001$), Kpasenkpe ($F_{3,12} = 23.12$; $p < 0.001$), and Settlement ($F_{3,12} = 44.82$; $p < 0.001$). In all test locations, damage was highest in the conventional cowpea entries and lowest in the Songotra T, which provided total protection (Table 2).

For the percentage of seed damage by LPBs, there were no significant location ($F_{3,48} = 1.54$; $p = 0.217$) effects when the data were combined and analyzed. There was, however, a significant cowpea entry effect ($F_{3,48} = 26.65$; $p < 0.001$) and location $\times$ cowpea entries ($F_{9,48} = 2.17$; $p = 0.041$) interaction effects. Again, Songotra T planted at all four locations recorded no seed damage at all sites, while Wang-Kae planted at Akukayili suffered the highest damage (Table 3).

Table 2. Damage to pods and seeds of different cowpea entries by pod-sucking bugs (PSBs) and legume pod borers (LPBs) in four different locations in northern Ghana.

Location	Entry	PSB-Damaged Pods/Plant	PSB-Damaged Seeds (%)	LPB-Damaged Pods/Plant
Akukayili	Kirkhouse Benga	1.9 (1.7 + 0.1) a	8.0 (3.0 + 0.1) a	1.1 (1.4 + 0.1) a
	Songotra	2.2 (1.8 + 0.1) a	16.8 (4.1 + 0.5) a	1.0 (1.4 + 0.1) a
	Songotra T	2.4 (1.8 + 0.1) a	19.0 (4.4 + 0.4) a	0.0 (1.0 + 0.0) b
	Wang Kae	1.8 (1.7 + 0.1) a	16.8 (4.1 + 0.5) a	1.0 (1.4 + 0.1) a
	<i>p</i> -value	0.459	0.108	0.009
Chinchang	Kirkhouse Benga	3.3 (2.1 + 0.1) a	6.3 (2.7 + 0.0) b	2.0 (1.7 + 0.1) a
	Songotra	3.3 (2.1 + 0.1) a	12.3 (3.6 + 0.2) ab	2.0 (1.7 + 0.0) a
	Songotra T	3.3 (2.1 + 0.1) a	15.5 (4.0 + 0.4) a	0.0 (1.0 + 0.0) b
	Wang Kae	3.0 (2.0 + 0.1) a	12.8 (3.7 + 0.3) ab	1.8 (1.7 + 0.1) a
	<i>p</i> -value	0.927	0.031	<0.001
Kpasenkpe	Kirkhouse Benga	2.7 (1.9 + 0.1) a	16.0 (4.1 + 0.3) a	1.7 (1.6 + 0.1) a
	Songotra	3.6 (2.2 + 0.1) a	40.8 (6.3 + 0.8) a	2.5 (1.9 + 0.1) a
	Songotra T	3.1 (2.0 + 0.2) a	32.0 (5.7 + 0.4) a	0.0 (1.0 + 0.0) b
	Wang Kae	2.9 (2.0 + 0.0) a	28.0 (5.3 + 0.4) a	2.0 (1.7 + 0.1) a
	<i>p</i> -value	0.420	0.054	<0.001
Settlement	Kirkhouse Benga	2.8 (2.0 + 0.1) c	35.5 (8.7 + 3.2) a	3.0 (1.8 + 0.1) a
	Songotra	3.6 (2.1 + 0.1) b	32.8 (7.2 + 1.0) a	3.4 (1.9 + 0.3) a
	Songotra T	5.1 (2.5 + 0.1) a	36.5 (6.4 + 0.7) a	0.0 (1.0 + 0.0) b
	Wang Kae	3.3 (2.2 + 0.1) bc	36.5 (6.3 + 0.7) a	2.9 (1.8 + 0.1) a
	<i>p</i> -value	<0.001	0.923	<0.001

Note: figures in brackets are square root [$\sqrt{(x + 1)}$] transformed means $\pm$ standard error of means; for each location, means followed by different letters are significantly different at 5% probability threshold.

Table 3. Interaction effects of location and cowpea entries on mean percentage of grain damage by legume pod borers (LPBs) in northern Ghana.

Location/Cowpea Entry	Akukayili	Chinchang	Kpasenkpe	Settlement	Mean
Kirkhouse Benga	3.5 (2.0 $\pm$ 0.4) bc	4.8 (2.4 $\pm$ 0.2) abc	4.8 (2.4 $\pm$ 0.1) abc	3.8 (2.0 $\pm$ 0.6) bc	4.2 (2.2 $\pm$ 0.2) A
Songotra	3.8 (2.1 $\pm$ 0.2) abc	2.3 (1.8 $\pm$ 0.1) bc	12.3 (3.5 $\pm$ 0.6) a	4.5 (2.3 $\pm$ 0.2) abc	5.7 (2.4 $\pm$ 0.2) A
Songotra T	0.0 (1.0 $\pm$ 0.0) c	0.0 (1.0 $\pm$ 0.0) c	0.0 (1.0 $\pm$ 0.0) c	0.0 (1.0 $\pm$ 0.0) c	0.0 (1.0 $\pm$ 0.0) B
Wang-Kae	6.3 (2.7 $\pm$ 0.5) ab	6.0 (2.6 $\pm$ 0.2) ab	4.5 (2.3 $\pm$ 0.2) abc	5.8 (2.6 $\pm$ 0.1) ab	5.6 (2.5 $\pm$ 0.1) A
Mean	3.4 (1.9 $\pm$ 0.2) A	3.3 (1.9 $\pm$ 0.2) A	5.4 (2.3 $\pm$ 0.3) A	3.5 (2.0 $\pm$ 0.2) A	Mean
<i>p</i> -values					
Location (L)	0.217				
Cowpea entry (C)	<0.001				
L $\times$ C	0.041				

Note: figures in brackets are square root [$\sqrt{(x + 1)}$] transformed means $\pm$ standard error of means; main effect (location or cowpea entry) means followed by different uppercase letters are significantly different at a 5% probability threshold; interaction effect means followed by different lowercase letters are significantly different at a 5% probability threshold.

3.1.4. Natural Predator Abundance

In this study, two predatory arthropods (spiders, *Oxyopes* sp., and ladybird beetles, *Coccinella* sp.) were recorded across the four testing sites. The abundance of spiders was significantly affected by the location ($F_{3,48} = 7.77$; $p < 0.001$). However, there was no significant cowpea entry effect for this variable at Akukayili ($F_{3,12} = 2.34$; $p = 0.125$), Chinchang ($F_{3,12} = 0.13$; $p = 0.939$), Settlement ($F_{3,12} = 0.59$; $p = 0.635$), or Kpasenkpe ($F_{3,12} = 0.30$; $p = 0.828$). The mean number of spiders per plant was 0.14 across locations.

Similarly, the number of ladybird beetles per plant was significantly affected by the location ($F_{3,48} = 10.38$; $p < 0.001$). Except for settlement ($F_{3,12} = 5.51$; $p = 0.013$), there was no significant cowpea entry effect for ladybird beetle abundance at Akukayili ($F_{3,12} = 2.00$; $p = 0.168$), Chinchang ($F_{3,12} = 0.85$; $p = 0.495$), or Kpasenkpe ($F_{3,12} = 0.81$; $p = 0.513$). The means of this variable at Akukayili, Chinchang, and Kpasenkpe were 0.03, 0.03, and 0.1, respectively. At Settlement, this predatory beetle was present in only Songotra (Table 4).

Table 4. Effect of cowpea entries on mean number of predatory ladybird beetles per plant at Settlement, Savannah Region.

Cowpea Entry	Mean No. of Ladybird Beetles/Plant
Kirkhouse Benga	0.0 (1.0 $\pm$ 0.5) b
Songotra	0.1 (1.0 $\pm$ 0.0) a
Songotra T	0.0 (1.0 $\pm$ 0.5) b
Wang-Kae	0.0 (1.0 $\pm$ 0.5) b
<i>p</i> -value	0.013

Note: figures in brackets are square root transformed means $\pm$ standard error of means; means followed by different letters are significantly different at 5% probability level.

3.1.5. Correlations Between Pest Infestations, Pod Damage, Grain Damage, and Grain Yield

In this study, LPB/20 flowers were positively correlated with LPB-damaged seeds ($p = 0.0076$) but negatively correlated with PSB-damaged seeds ($p = 0.0095$). Similarly, the PSBs/m was positively correlated with PSB-damaged seeds ($p = 0.0126$). There was a negative correlation between thrips/20 flowers and LPB-damaged pods ($p = 0.0450$) (Table 5).

Table 5. Correlation between legume pod borers (LPBs)/20 flowers, thrips/20 flowers/pod-sucking bugs (PSBs)/plant, LPB damage to pods and grains, PSB damage to pods and grains, and grain yield.

	LPB-Damaged Seeds	PSB-Damaged Seeds	Grain Yield	LPBs/20 Flowers	LPB Damaged Pods	PSB-Damaged Pods	PSBs/Plant	Thrips/20 Flowers
LPB-damaged seeds	-							
PSB-damaged seeds	0.129 ns							
Grain yield	−0.322 **	0.030 ns						
LPB/20 flowers	0.331 **	−0.322 **	−0.314 **					
LPB-damaged pods	0.458 **	0.098 ns	−0.311 *	0.304 **				
PSB-damaged pods	−0.025 ns	0.332 **	0.331 **	−0.200 ns	0.143 ns			
PSBs/m	−0.084 ns	0.310 *	−0.008 ns	0.246 ns	0.057 ns	−0.073 ns		
Thrips/20 flowers	−0.001 ns	−0.113 ns	0.041 ns	−0.080 ns	−0.252 *	−0.135 ns	0.109 ns	-

Note: LPB = legume pod borer; PSBs = pod-sucking bugs; * = significant at 5% probability threshold; ** = significant at 1% probability threshold. ns = not significant at 5% probability threshold.

There was a positive correlation between PSB-damaged seeds and PSB-damaged pods ($p = 0.0073$). Also, LPB-damaged seeds were positively correlated with LPB-damaged pods ($p < 0.001$) (Table 5). As expected, grain yield negatively correlated with LPB/20 flowers ($p = 0.0115$), LPB-damaged pods ($p = 0.0124$), and LPB-damaged seeds ($p = 0.0053$) but positively correlated with PSB-damaged pods ($p = 0.0075$) (Table 5).

3.2. Grain Yield

A combined-site analysis of yield showed no significant location ($F_{3,48} = 1.07$; $p = 0.372$) or location $\times$ cowpea entry ($F_{9,48} = 1.16$; $p = 0.343$) interaction effects. However, cowpea entry ($F_{3,48} = 12.88$; $p < 0.001$) significantly affected the yield. The mean yield was highest in Songotra T (2538.80 kg/ha), while Kirkhouse Benga (1413.60 kg/ha) was lowest. There were no significant differences between Kirkhouse Benga, Wang-Kae, and Songotra in terms of their yield (Figure 2).

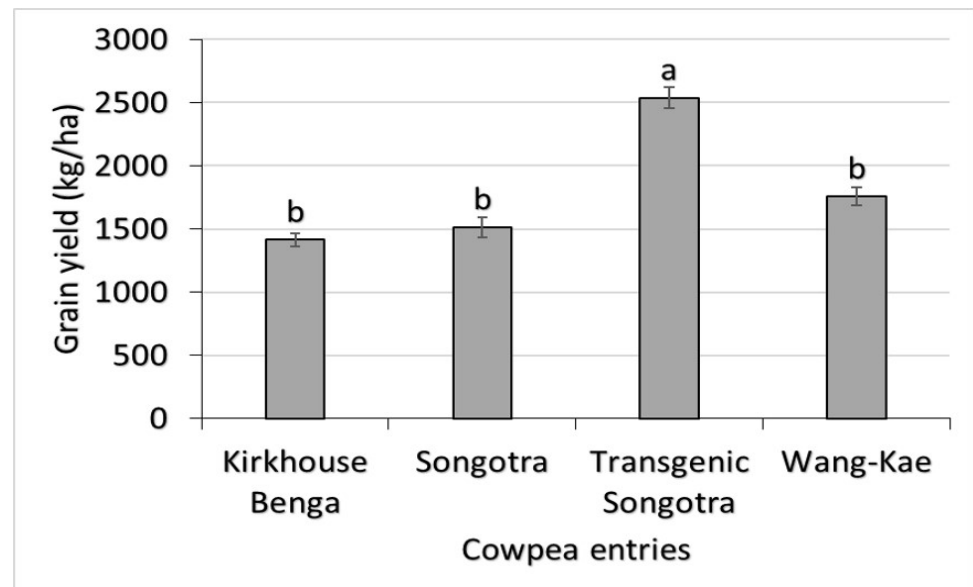


Figure 2. Grain yield (kg/ha) of different cowpea entries averaged across four locations in northern Ghana. Note: bars are means $\pm$ standard error of means; bars followed by different letters are significantly different at a 5% probability threshold.

3.3. Economic Analysis

The total cost of producing a hectare of cowpea under researcher-managed conditions and using a maximum of two insecticide applications remained constant at USD 461.93/ha, irrespective of the chosen entry, as there was no market premium based on the chosen cowpea. However, this production cost could increase to at least USD 534.64/ha under farmer management conditions, where at least six rounds of insecticide sprays are applied before harvest. Insecticides and their application costs account for the high cost of production under farmer management practices (Table 6).

The return on investment estimates showed that cowpea cultivation is generally profitable. Ranked in ascending order for the entries studied, the varieties with the highest return on investment were Kirkhouse-Beng, Songotra, Wang-Kae, and Songotra T. The variety Songotra T had the highest return on investment because of its high yield compared to the other varieties under a maximum of two rounds of insecticide sprays. Using data from the national average yield of cowpea, irrespective of the conventional variety grown, the return on investment was estimated to be 201.44%. This is in spite of the fact that at least six rounds of insecticide spray are applied under farmer management practices (Figure 3). On average, for every USD 1.00 invested, farmers earn between USD 2.14 (for Kirkhouse-Benga) and USD 4.64 (for Songotra T) (Figure 3).

Table 6. Return on investment for various cowpea entries studied.

Variable	Kirkhouse- Benga	Songotra	Wang-Kae	Songotra T	All Conventional Varieties ¹
Average grain yield (kg/ha)	1413.60	1513.20	1756.80	2538.80	1570.0
Grain price (USD/kg)	1.03	1.03	1.03	1.03	1.03
Total revenue (USD/ha)	1451.09	1553.33	1803.39	2606.12	1611.63
Production cost (USD/ha)					
Land rental	47.05	47.05	47.05	47.05	47.05
Cost of seeds	32.08	32.08	32.08	32.08	32.08
Cost of ploughing	47.05	47.05	47.05	47.05	47.05
Cost of planting	64.16	64.16	64.16	64.16	64.16
Herbicide application	12.83	12.83	12.83	12.83	12.83
Weed management	42.77	42.77	42.77	42.77	42.77
Insecticide application cost	25.66	25.66	25.66	25.66	76.99
Insecticide cost	10.69	10.69	10.69	10.69	32.08
Pre- and post-emergence herbicide cost	23.52	23.52	23.52	23.52	23.52
Harvesting cost	64.16	64.16	64.16	64.16	64.16
Threshing cost	53.46	53.46	53.46	53.46	53.46
Winnowing and bagging	38.49	38.49	38.49	38.49	38.49
Total product cost (USD/ha)	461.93	461.93	461.93	461.93	534.64
Net benefit (USD/ha)	989.15	1091.39	1341.45	2144.19	1076.99
Return on investment	214.13%	236.27%	290.40%	464.18%	201.44

Notes: ¹ Estimated using national average yield/ha of all conventionally grown cowpeas in Ghana in farmers' fields [30].

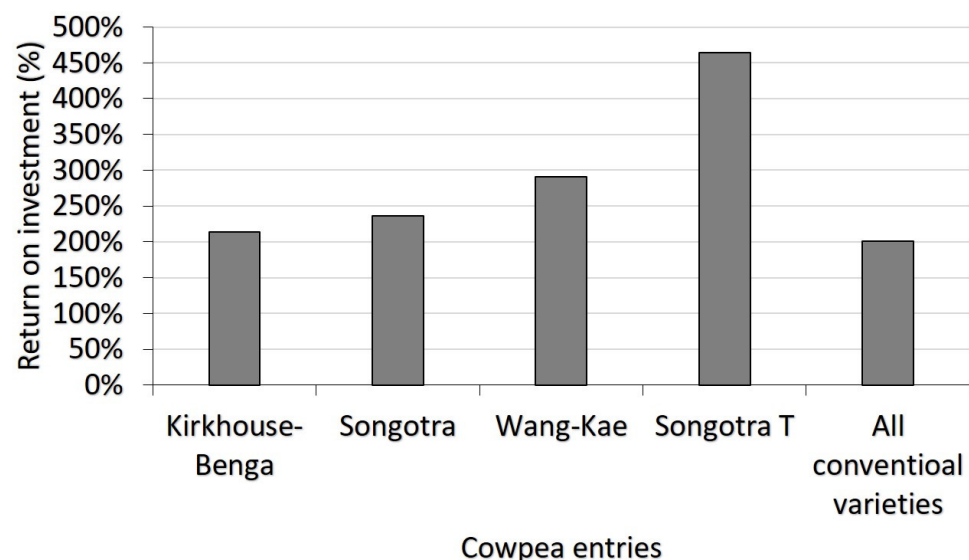


Figure 3. Return on investment of cowpea varieties grown in northern Ghana. Note: return on investment for “all conventional varieties” was estimated using average yields of the crop in farmers’ fields in Ghana [30].

4. Discussion

The economic threshold for managing LPBs is estimated to be one larva per meter row [35]. Damage to pods and grains is caused by third to fifth instar larvae, which bore

into the pods and consume the developing grains [33]. In this study, pod and grain damage caused by LPBs was greater in the conventional cowpea varieties, and this was because larval infestations were generally at or above the established economic threshold. To date, there is no known commercially cultivated non-transgenic cowpea variety that has resistance to LPBs [13,15]. This may partly be because it has not been possible to transfer resistance genes from wild cowpeas, which possess some level of resistance to LPBs, to cultivated cowpea [20,21]. Thus, the observed susceptibility of conventional cowpea varieties to LPB damage was not unexpected. Cry1Ab insecticidal protein expression in the reproductive parts [22] of Songotra T (also known as Sampea 20-T in Nigeria) explains its resistance to damage by LPBs.

The efficacy of cowpea expressing Cry1Ab insecticidal protein against LPBs under screenhouse conditions in confined field trials and using laboratory-reared LPB neonates was already known [19]. According to [33], the flower bud stage is the most preferred for oviposition, and, at this stage, young larvae substantially damage and reduce the potential of the crop to form pods. A single LPB larvae may consume four to six flowers before larval development is completed. In this study, LPB infestations were mainly in flowers of the conventional cowpea varieties, but none were found in flowers randomly sampled from the Songotra T plots. This is consistent with results of earlier studies, which reported excellent protection of transgenic cowpeas from LPBs [25]. The cry proteins protect crops when ingested by lysing midgut epithelial cells and forming pores, typically leading to feeding reduction or cessation and eventual death of susceptible insects [23,24]. Adults of LPBs probably oviposited on Songotra T, but the newly emerged neonates died after their first attempted ingestion of floral or vegetative tissues of the transgenic plants. This is because the *cry1Ab* gene is active in the vegetative and reproductive parts of cowpea [22].

The performance of Songotra T in the presence of other economically important field pests of cowpea and under typical cowpea growing conditions was not documented until now. Here, Songotra T was found to be as susceptible as conventional cultivars to the non-lepidopteran pests of cowpea, such as whiteflies, leafhoppers, thrips, and the PSB complexes. The lack of toxic activity of the Cry1Ab protein against the non-lepidopteran pests of cowpea reported in this study is corroborated by Ba et al. (2018) [36], who indicated that this protein is specific to lepidopteran pests only. Hence, the introgression of the *cry1Ab* gene into the commercial cowpea variety Songotra did not confer resistance to other field pests of cowpea, and thus cultivation of Songotra T must be accompanied by strategies to mitigate the damaging effects of these pests.

Pod-sucking bugs (PSBs) are reported to cause 40–80% of damage in cowpea, and this pest complex, together with LPBs, are recognized as key reproductive stage pests of cowpea [7]. Hence, this caused the observed lack of difference between the Songotra T and conventional cowpea entries in terms of PSB damage to pods and grains, except at Settlement for pods and Chinchang for grains. At Settlement and Chinchang, the differences among varieties for pod and grain damage were mainly driven by higher pod and grain yield in Songotra T compared to the other varieties. These findings emphasize the need to adopt mitigation strategies for PSBs when Songotra T is cultivated, and many studies already show that a single round of insecticide spray at the podding stage is adequate for protecting cowpea from this pest complex [6,7,37,38].

Across the four test locations, spiders and ladybird beetles were the most abundant predators recorded. In general, we did not find differences in abundance of these predators between Songotra T and the other cowpea varieties. Pellegrino et al. (2018) [39] reported no adverse effects of *Bacillus thuringiensis* (Bt) crops on species in the non-target families Araneidae, Chrysopidae, and Coccinellidae. Similar conclusions were made by Ba et al. (2018) [36] with regards to the effect of Cry1Ab on non-target organisms, such as spiders and ladybird beetles. In the current work, all entries were sprayed two times with chemical insecticides, so the results are comparable. However, in typical cowpea farmers' fields, the spraying regimes would typically range between 6 and 10 sprays per season, thus having

a huge negative impact on the population of beneficial insects. As mentioned above, the recommended spraying regime for Songotra T is just two sprays per season.

As expected, the correlation analysis showed that greater LPB infestations were linked to increased damage to pods and grains and, consequently, reduced yields. This is consistent with the findings of Sodedji et al. (2020) [40], who reported that LPB infestations and damage in cowpea can lead to complete crop failure. Similarly, greater PSB infestations corresponding to increased damage were detected in this study. In contrast, thrips infestations did not result in significant yield reductions. These correlation outputs confirm the results of studies that found PSBs and LPBs to be the most important pests in cowpea [9,41,42]. Infestation thresholds for thrips are pegged at seven thrips per flower [8]. Because thrips infestation figures reported in this work did not exceed the threshold, the correlation coefficients were not significant between this pest and grain yields. Thus, the below-threshold abundance of thrips in cowpea-producing areas in northern Ghana makes LPBs the most economically important pest at flowering.

In general, grain yields were approximately two-fold higher in Songotra T compared to the conventional cowpea entries. This translated into a return on investment of over 450% for Songotra T compared to the 200 to 291% returns recorded for the conventional varieties. In real farmers' fields where conventional varieties are cultivated, the higher number of insecticide applications used per season further increases the return gap between the conventional varieties and Songotra T, which only needs two sprays.

To sustain the high yields and financial returns of Songotra T, there is a need to consider developing cowpea varieties that express more than one insecticidal protein against LPBs. This is because of the possibility of LPBs developing resistance to the Cry1Ab protein over time [43]. In other crops, pest resistance development has been managed through pyramiding genes expressing different insecticidal proteins [44–46]. This option to improve the durability of Songotra T is already in progress, and transgenic cowpeas containing the *cry2Ab* gene, which provides protection against LPB, have been tested successfully in confined field trials in Nigeria, Ghana, and Burkina Faso. The *cry2Ab* transgenic cowpea (event AAT-Ø245F-3) was produced through *Agrobacterium-tumefaciens*-mediated transformation, as described for *cry1Ab*, and it has been approved for environmental release in Ghana and Nigeria. As for other Bt proteins, the Cry2Ab toxin from this gene binds to a receptor on the midgut of insects, inserting itself into the membrane and forming pores that kill the cell, eventually leading to the death of the pest [47,48]. Work is therefore currently ongoing to combine the *cry1Ab* and *cry2Ab* genes to generate an improved variety with long-lasting resistance to the LPB.

5. Conclusions

This work showed that modern biotechnology can be used to enhance nutrition and reduce food insecurity in SSA and other regions of the world by increasing the grain yield of a major staple food while mitigating hazardous consequences of excessive insecticide use in its cultivation. The introgression of the *cry1Ab* gene conferred Songotra T with complete resistance to LPBs, resulting in an approximately one-and-a-half-fold yield increment compared to conventional cowpeas. Consequently, the financial returns of cultivating Songotra T were higher than those for the other cowpea varieties. The Cry1Ab had no lethal effects on non-lepidopteran field pests of cowpea, such as thrips, leafhoppers, and PSBs, and, most importantly, it had no effect on predators present. Growers of this variety will still need to adopt integrated pest management packages that effectively mitigate damage by thrips and PSBs. To delay the development of resistance by LPBs to the Cry1Ab protein, an effective insect resistance management package will be developed before deploying the seed to farmers.

Author Contributions: Conceptualization, J.A.N., G.A.A. and E.Y.O.; methodology, J.A.N., G.A.A., E.Y.O., P.A., I.A., F.N.O. and J.B.T.; validation, J.B.T., D.J.M., J.M.B. and T.J.V.H.; formal analysis, J.A.N., G.A.A., P.M.E., D.J.M., J.M.B. and T.J.V.H.; investigation, G.A.A., E.Y.O., P.A., I.A., F.N.O., A.S., S.L., M.Z., J.Y.K. and H.K.A.; resources, I.A., F.N.O. and J.B.T.; data curation, P.A., J.Y.K. and H.K.A.; writing—original draft preparation, J.A.N., P.M.E., J.M.B. and T.J.V.H.; writing—review and editing, G.A.A., E.Y.O., P.A., A.S., S.L., M.Z. and D.J.M.; supervision, A.S., S.L., M.Z., J.M.B. and T.J.V.H.; project administration, J.A.N., I.A., F.N.O., D.J.M., J.M.B. and T.J.V.H.; funding acquisition, I.A., F.N.O. and J.B.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by a United States Agency for International Development (USAID) Grant (AID-OAA-A-14-00035) received through the African Agricultural Technology Foundation (AATF), Nairobi, Kenya.

Data Availability Statement: The data used for this paper are available upon written request to the corresponding author.

Acknowledgments: The authors acknowledge the African Agricultural Technology Foundation (AATF) for coordinating and providing funding for the Pod Borer Resistant Cowpea (PBRC) project in Ghana. The authors also thank the United States Agency for International Development (USAID, AID-OAA-A-14-00035) for providing funds and support for the PBRC project. We are also grateful to the staff and management of the CSIR–Savanna Agricultural Research Institute, especially Fredrick Anaman, Mohammed Abdulai, Abdul-Rashid Seidu, Abdulai Suweiba, and Rebecca Kaba, for their field technical assistance.

Conflicts of Interest: The authors declare no conflicts of interest.

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