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Abstract

Cowpea (*Vigna unguiculata* spp *unguiculata*) is adapted to the drier agro-ecological zones of West Africa where it is a major source of dietary protein and widely used as a fodder crop. Improving the productivity of cowpea can enhance food availability and security in West Africa. Insect predation – predominately from the legume pod borer (*Maruca vitrata*), flower thrips (*Megalurothrips sjostedti*) and a complex of pod-sucking bugs (e.g., *Clavigralla* spp) – is a major yield-limiting factor in West African cowpea production. Dramatic increases in yield are shown when *M. vitrata* is controlled with insecticides. However, availability, costs, and safety considerations limit pesticides as a viable option for boosting cowpea production. Development of Bt-cowpea through genetic modification (GM) to control the legume pod borer is a promising approach to cowpea improvement. Cowpea expressing the lepidopteran-active Cry1Ab protein from *Bacillus thuringiensis* being developed as a first generation Bt-cowpea crop for West Africa. Appropriate stewardship of Bt-cowpea to assure its sustainability under West African conditions is critical to its successful development. A first step in this process is an environmental risk assessment to determine the likelihood and magnitude of adverse effects of the Cry1Ab protein on key environmental protection goals in West Africa. Here we describe the results of an expert panel convened in 2009 to develop the problem formulation phase for Bt-cowpea and to address specific issues around gene flow, non-target arthropods, and insect resistance management.

Keywords

Agronomy, Biosafety, Environmental risk assessment, Risk assessment, Non-target organism, Gene flow, IRM

Disciplines

Agricultural Science | Agriculture | Agronomy and Crop Sciences | Entomology | Genetics | Plant Breeding and Genetics

Comments

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Regulatory considerations surrounding the deployment of Bt-expressing cowpea in Africa

Report of the deliberations of an expert panel

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Keywords: biosafety, environmental risk assessment, risk assessment, non-target organism, gene flow, IRM

Cowpea (*Vigna unguiculata* ssp. *unguiculata*) is adapted to the drier agro-ecological zones of West Africa where it is a major source of dietary protein and widely used as a fodder crop. Improving the productivity of cowpea can enhance food availability and security in West Africa. Insect predation—predominately from the legume pod borer (*Maruca vitrata*), flower thrips (*Megalurothrips sjostedti*) and a complex of pod-sucking bugs (e.g., *Clavigralla* spp)—is a major yield-limiting factor in West African cowpea production. Dramatic increases in yield are shown when *M. vitrata* is controlled with insecticides. However, availability, costs and safety considerations limit pesticides as a viable option for boosting cowpea production. Development of Bt-cowpea through genetic modification (GM) to control the legume pod borer is a promising approach to cowpea improvement. Cowpea expressing the lepidopteran-active Cry1Ab protein from *Bacillus thuringiensis* is being developed as a first generation Bt-cowpea crop for West Africa. Appropriate stewardship of Bt-cowpea to assure its sustainability under West African conditions is critical to its successful development. A first step in this process is an environmental risk assessment to determine the likelihood and magnitude of adverse effects of the Cry1Ab protein on key environmental protection goals in West Africa. Here we describe the results of an expert panel convened in 2009 to develop the problem formulation phase for Bt-cowpea and to address specific issues around gene flow, non-target arthropods and insect resistance management.

Introduction

Cowpea (*Vigna unguiculata* ssp. *unguiculata*) ((L.) Walp.) is a leguminous African crop that provides high-quality protein-rich food for people, fodder for livestock and nitrogen for the soil, all three of which are in short supply in Africa. Also known as black-eyed pea, Southern pea, frijole, lobia, feijao caupi and niebe, cowpea is highly adapted to the hot and sparse rainfall climates of the Sahelian and Sudanian zones in Africa. About 80% of the world's cowpea growing area is in Africa.¹ In Nigeria, the largest cowpea producing country in Africa, nearly 80% of the crop is grown in the semi-arid north.² In West Africa, where it likely originated as an agricultural crop, cowpea is the most economically important legume.¹ It was likely domesticated and spread

as a crop together with sorghum and pearl millet.³ Cowpea is mainly grown by low-resource farmers who prize it for its ability to yield well on poor soil with a minimum of moisture. Because cowpea provides excellent ground cover it helps to preserve moisture in the semi-arid zones. As a nitrogen-fixing legume it is important for soil fertility.⁴ In addition to providing food and fodder at the subsistence level it is also a cash crop sold by women growers in local markets and by increasing numbers of commercial farmers in the regional trade system. Hundreds of thousands of tons of cowpea grain move along ancient trade routes from the semi-arid northern regions of West Africa to the rapidly growing coastal mega-cities like Accra, Lagos and Abidjan. Per capita cowpea consumption is very high in West Africa and similar to common bean consumption in Latin America. Although

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largely undeveloped, there are potential export markets in South America and Europe as well.

Cowpea is an important economic crop in part because it is highly nutritious and also because virtually the entire plant is edible. The grain, the green pods, the dried leaves and hay all command good market prices. One factor driving demand is the high-quality protein it offers. Both the grain and dried foliage contain about 23–25% protein by weight.⁵ In many parts of Africa, fresh tender green cowpea leaves picked before flowering are the first part of the cowpea crop harvested, followed by the fresh grains in slightly immature pods. These leaves and the fresh-shelled grains provide needed protein during the period Africans call the “hungry time” (termed “lokotchin yinwa” in the Hausa language). This is the period when the harvest of the previous year has been sold or consumed and food is scarce before the next harvest. In the Sahel, cowpea hay often commands high prices. In Senegal in recent years green pods have become popular as a cash crop with women selling basins of green pods along the roadsides when the pods are maturing in the fields. In addition to protein, cowpea grain is an excellent source of bulk carbohydrate (CHO), roughly 60% CHO by weight, and is nearly as good as cereals. Cowpea grain also offers key vitamins including thiamin, riboflavin, ascorbic acid, niacin and folic acid. It is low in fat (1–2% by weight), and it represents a good source of fiber at about 6%.⁶ It is relatively low in sulfur amino acids but high in lysine and other essential amino acids, making it a good complement to the mainly cereal diets of many Africans. Thanks to the nutrition it offers, cowpea has been considered by the US National Aeronautics and Space Administration as a possible space station crop.⁷

Insects are the major cause of crop loss in cowpea.⁸ In some years grain yields can be reduced to nearly zero if the crop is not sprayed with insecticide. Insects are one of the major constraints to wider cultivation of cowpea in the more Southern, moister regions of West Africa. Major insect pests include aphids (*Aphis craccivora* Koch; Hemiptera: Aphidae), flower thrips (*Megalurothrips sjostedti* Tryborn; Thysanoptera: Thripidae), the legume pod borer (*Maruca vitrata* Fabricius; Lepidoptera: Crambidae), and a complex of pod sucking bugs (*Clavigralla* spp; Hemiptera: Coreidae) which collectively cause substantial yield losses. In northern Nigeria, for example, untreated cowpea plots yielded 76 kg ha⁻¹, while fields treated with insecticide yielded 1,382 kg ha^{-1,2}. The insect problems of cowpea are compounded by the fact that insecticides not labeled for cowpeas (e.g., cotton insecticides) are often used on them in the field as well as in storage, where bruchid beetles (*Callosobruchus maculatus* Fabricius; Coleoptera: Bruchidae) are the major pest post-harvest.⁹ In some areas of West Africa, multiple sprays are used each season. The negative ecological and health consequences of insecticide use are a growing concern to farmers, scientists, extension agents and policymakers.¹⁰ Traditional protection methods and chemical insecticides have largely failed to stop insect-caused losses.⁹ In addition to their high cost and uncertain availability, insecticides require sprayers, proper protection practices and training to be effective and safe.

One of the most serious pests of cowpea is the legume pod borer (*M. vitrata*; LPB). Cowpea cultivars are generally all susceptible to the LPB and thus far, only low levels of resistance have been reported in germplasm screens.⁸ A proven screening technique for detecting resistance to the LPB is available but no known cultivar has been identified with more than weak resistance to this destructive insect.¹¹ Interestingly, one relative of the cowpea, *V. vexillata*, is highly resistant to the LPB but the basis of the resistance is thought to be due to the natural plant chemical para-amino phenyl alanine (PAPA), which is likely detrimental to humans when consumed.¹² In addition, it has not been possible to hybridize cultivated cowpea and *V. vexillata* despite attempts over many years by several investigators. For these reasons naturally-occurring resistance genes cannot be introgressed into cowpea by conventional breeding techniques.¹³ Although insecticides can be used to control of LPB, the timing of the treatments is critical because LPB, like other pests in the insect family Crambidae, feeds internally in the plant and is thus shielded from externally-applied insecticides.

One solution proven useful for control of boring insects in the family Crambidae is crop biotechnology.^{9,14-16} A public sector initiative to introduce insect resistance into cowpea was begun at Purdue University in 1987 and this effort was subsequently joined by several organizations, particularly CSIRO, Australia, NGICA (The Network for the Genetic Improvement of Cowpea for Africa), the AATF (African Agriculture Technology Foundation), the Monsanto Co., the Rockefeller Foundation and USAID. The current cowpea project aims to develop a transgenic Bt-cowpea variety for control of LPB.^{9,17} Confined field trials of the first Bt-cowpea events conducted in 2009 and 2010 in Nigeria established that Cry1Ab-expressing cowpea plants have significantly reduced feeding damage due to LPB. Thus, Bt-cowpea has the potential to reduce losses to LPB as well as substantially reduce the growing insecticide load applied to cowpea in an attempt by farmers to preserve yield and harvest quality.

A critical step in the Bt-cowpea development process is the submission of a pre-market regulatory safety package to the relevant African regulatory authorities in countries where Bt-cowpea will be grown.¹⁸⁻²⁰ Since many countries in the cowpea growing region of West Africa are also signatories to the Cartagena Protocol on Biosafety to the Convention on Biological Diversity the dossiers will have to address issues unique to the treaty particularly in the area of liability and redress.²¹ The regulatory package will address two broad areas: (i) food and feed safety and (ii) environmental safety. The environmental safety of Bt-cowpea, the subject of this paper, is evaluated through an internationally recognized process called environmental risk assessment (ERA). The ERA of all GM plants, including Bt-cowpea, is designed to answer very specific, relevant and realistic questions about the potential risks of introducing those plants into the environment.^{19,22-24}

The ERA process includes three main phases, namely, problem formulation, analysis (data collection) and risk characterization.^{20,22,25-27} In the problem formulation phase the protection goals are identified (e.g., the protection of beneficial insects or wild plant relatives). The information that is considered during problem formulation comes from the published scientific

literature, stakeholders, the developers of the technology and, importantly, expert opinion.¹⁹ Although protection goals are based on the social, cultural, economic and environmental objectives of a particular country,²⁸ there are some general environmental attributes, such as biodiversity and agricultural sustainability, that are routinely assessed worldwide for risks posed by insect-resistant GM crops. The means by which these crops could harm these attributes include: (i) gene flow to wild relatives, (ii) the potential effects on non-target organisms (NTOs) (primarily arthropods) and (iii) insect resistance management (IRM).²⁹⁻³¹ Problem formulation should first result in a conceptual model that describes how harm from the introduced transgene can occur. The end goal of problem formulation is to generate testable scientific hypotheses and effective tests of those hypotheses that are relevant to regulatory decision-making. These are then addressed in the analytical phase of the risk assessment.^{20,22,25-27} Finally, an analysis plan is constructed that is consistent with the risk hypotheses and which establishes the relationship between the transgene and the ecological impacts of concern.¹⁹

To address key issues associated with the environmental safety of Bt-cowpea an international panel of experts was convened at the Donald Danforth Plant Science Center in St. Louis, Missouri, USA, March 2–6, 2009. The panel was tasked with addressing the problem formulation phase of the ERA. The approach taken was to present the panel with a series of questions intended to elicit discussion in the following topic areas:

- The impact of gene flow from Bt-expressing cowpea into wild relatives of cowpea;
- The impact of Bt-expressing cowpea on non-target organism populations; and
- The potential for target pest populations to evolve resistance to the Bt protein.

For each topic area, the panel was asked to provide in-depth opinion as follows:

- Consider currently available information;
- Identify hazards and determine appropriate assessment endpoints;
- Determine data gaps and devise experimental strategies to collect the necessary data;

The deliberations and recommendations of that expert panel on issues related to the environmental safety of Bt-cowpea are summarized below.

Panel Discussion: Gene Flow in Cowpea

Gene flow between cultivated plants and their wild relatives is a continuous natural phenomenon not unique to GM crops.^{32,33} When GM crops are grown in proximity to compatible relatives, risk assessment should be focused on the consequences of an introduced allele, particularly when the introduced trait is one that may confer a selective advantage.^{29,32-40} Similar to that described for maize and sorghum,^{34,35} cowpea exists in West Africa as a series of landraces that interbreed with one another and which are continually being modified by farmers.⁹ In addition, wild cowpea exists outside of cultivation. A key first issue is whether gene flow can occur with any reasonable frequency between cultivated GM

cowpea and other cultivated non-GM varieties, land races, wild cowpeas or other related *Vigna* species.³³ If the answer is yes, then the next stage of the risk assessment will be to determine if the resulting transfer of the *cry1Ab* transgene would have any effect on the safety of food, feed or the environment. As mentioned below, the food and feed safety of Cry1Ab is well established.¹⁶ For the environmental assessment the questions to address are whether the resulting hybridized plants are weedy or have an effect on non-target organisms, predominantly arthropods (addressed elsewhere in this paper). Effects on the biodiversity of both plants and animals²¹ and the potential for insect resistance must also be considered (also addressed elsewhere in this paper). Finally, in some jurisdictions co-existence of GM and non-GM crops is considered because these require separate production paths.²³

Question 1: What is the potential for the transgene to escape from Bt-cowpea and persist in sexually compatible cowpea in Africa and are additional studies needed to address this question?

Based on the information currently available and presented to the panel, the panel concluded that gene flow from cultivated cowpea (*Vigna unguiculata* ssp. *unguiculata*) to 'wild cowpea' (*Vigna unguiculata* ssp. *unguiculata* var. *spontanea*) will occur where wild cowpea is found in proximity to cultivated cowpea in West Africa. Most studies to date show that there is gene flow from cultivated cowpea to wild cowpea, possibly even >1%^{36,76} which, by expert opinion, is evolutionarily and ecologically significant.^{37-40,42} In addition, the transgene is likely to persist in the wild populations. Gene flow to other subspecies or species is not a concern since cultivated cowpea cannot interbreed with other *Vigna* species.⁴³

Question 2: What are the potential negative impacts that might result from the escape of the Bt gene into wild cowpea and how likely are these to occur?

(1) *Loss of wild type alleles through genetic swamping.* The panel concluded that this harm is not likely. 'Genetic swamping' can occur if the frequency of gene flow from the cultivated crop into a wild population is high enough that the wild populations become genetically uniform with the cultivated crop. Selection is not necessary for genetic swamping to occur, but there would need to be an increase in the level of hybridization between cultivated and wild cowpea beyond the current levels. Even when cowpea plantings are large, gene flow to wild cowpea would only be high enough to have a swamping effect at the very margins of cowpea fields. This would not be enough to alter the diversity in the majority of wild cowpea populations.

(2) *Loss of genetic variation in wild cowpea through selective sweep.* If there were strong selection for wild cowpeas carrying the Bt gene, in combination with linkage disequilibrium due to the predominately selfing mating system in cultivated cowpea, there could be selection for large portions of the cowpea genome linked to the Bt gene, replacing the genetic variation in the wild cowpea with genes from the cultivated cowpea.

(3) *Loss (reduced abundance) of a valued species.* If the Bt gene confers a selective advantage that increases the abundance of wild cowpeas in populations in non-agricultural habitats to a level that wild cowpea outcompetes other plant species, there could be a reduction in the abundance of the other valued plant species. The panel concluded that wild cowpea currently has low

invasive tendencies unrelated to LPB pressure so this harm would be unlikely to occur.

(4) *Loss of 'ecosystem services'*. If there is an increase in wild cowpeas (as above) to the point where the species became invasive, there could be a reduction in other resources that service the ecosystem, such as soil nutrients, water and light.

(5) *Loss of crop yield and quality*. If there is an increase in wild cowpeas in agricultural fields, there could be a reduction in both crop yield and quality, particularly if wild cowpea seeds are mixed with cowpea seeds at harvest. The panel thought this unlikely because seed used for planting is typically hand-selected with care and weedy hybrids are easy to recognize and are typically removed by the farmer.

Question 3: What information can be used to effectively predict whether these potential negative environmental consequences following gene flow from Bt-cowpea will or will not occur and are additional studies needed to address these questions? The panel developed a conceptual model comprised of a series of events (scenario) that must occur for cultivation of the Bt-cowpea crop to cause environmental harm due to gene flow.⁴⁴ This model is also relevant to later discussions of potential effects on non-target organisms and insect resistance management. A series of risk hypotheses were formulated to test whether 'at least one of the events necessary for harm will be absent'. Some hypotheses were corroborated with existing information, but others would require further experimentation. Using this approach it was possible to determine whether a harmful effect was not likely by demonstrating that any one of the events in the conceptual model was not likely. Based on the panel's discussion, a series of events (a conceptual model) that would lead to identified harmful effects could be as follows:

(1) Hybridization between the crop and the wild cowpea must occur;

(2) The transferred Bt trait increases the LPB resistance of the wild cowpea;

(3) The LPB (or other lepidopteran insects) is a pest of the wild species;

(4) The infested GM plants produce more seed than infested non-GM plants;

(5) An increase in seed production leads to an increase in abundance of wild cowpea carrying the Bt gene (selection).

(6) An increase in the abundance of wild cowpea reduces the:

- (a) abundance of a valued species;
- (b) resources that service the ecosystem;
- (c) crop yield and quality.

Given this fact pattern the panel considered what hypotheses could be tested to determine if these events would occur and whether existing information was sufficient or additional information would be necessary:

(1) *Hybridization between the crop and the wild cowpea. Hypothesis: Hybridization between the crop and the wild species does not occur.* Based on existing information, the panel determined that hybridization is likely to occur.

(2) *GM trait increases the insect resistance of the wild cowpea. Hypothesis: The Bt gene does not increase resistance in wild cowpea.*

Based on existing information, the panel determined that levels of insect resistance comparable to that which would be conferred by the Bt gene do not occur naturally in wild cowpea. Therefore, it is likely that the Bt gene will increase resistance to lepidopteran insect pests in wild cowpea.

(3) *The insects are a pest in the wild species. Hypothesis: Insects controlled by the Bt gene do not infest wild cowpea.* It is not clear from existing information whether lepidopteran insects that would be susceptible to the Bt Cry protein infest wild cowpea, including the target pest (LPB), although it seems likely that the pest complex in wild cowpea is the same as cultivated cowpea. It would be possible to test this hypothesis by surveying the insect pests present in wild cowpea populations.

(4) *Infested GM plants survive longer and produce more seed than infested non GM plants. Hypothesis: Insect infestation does not reduce plant survival or seed production.* If insects that infest wild cowpea are susceptible to the Bt protein, it is still possible that these insects do not decrease survival or reduce seed production in wild populations, and so do not limit the wild cowpea populations. If this is the case, the increased resistance in the wild populations would not correlate with a selective or competitive advantage in the wild cowpea. It would be possible to test this hypothesis by comparing survival and seed production in wild cowpea sprayed with a Bt-insecticide (a broad spectrum insecticide could also be used and would be the most conservative choice), to mimic the action of the Bt gene, to unsprayed wild cowpea. If survival and seed production in the unsprayed, insect-infested plants is the same as the sprayed plants, this would suggest that insects susceptible to the Bt protein do not reduce survival or seed production in wild cowpea.

(5) *An increase in seed production leads to an increase in abundance of wild cowpea carrying the Bt gene (selection). Hypothesis: An increase in seed production does not result in an increase in the abundance of wild cowpea.* If plants that are resistant to lepidopteran insect infestation produce more viable seeds, it is still possible that the number of seedlings that emerge from these seeds and survive to reproduce will not be increased. In this case, the number of seeds produced does not limit the wild populations. This could be tested by observing the number of seeds that survive to reproduce in plots sown with increasing seed densities. If the number of reproductive adults is not dependent on the number of seeds sown, this would suggest that the presence of the Bt gene will not correlate with a selective or competitive advantage in the wild cowpea.

(6) *An increase in the abundance of wild cowpea reduces:*

- (a) the abundance of a valued species;
- (b) resources that service the ecosystem;
- (c) crop yield and quality.

Hypothesis: An increase in the abundance of wild cowpea does not lead to the aforementioned identified harmful effects. If an increase in survival and reproduction due to increased insect resistance does result in an increased abundance of wild cowpea with the Bt gene, it is still possible that the more abundant wild cowpeas will not outcompete another valued species or reduce resources or reduce crop yield and quality. If it is likely that wild cowpeas will increase in abundance or there will be selection for wild cowpeas

with the Bt gene, then it might be necessary to design experiments to test further hypotheses.

Question 4: What is the potential risk from gene flow associated with the conduct of a limited scale (e.g., 10 acres or less) confined field trial (CFT) with Bt-cowpea when conditions to maintain genetic and material confinement (e.g., appropriate isolation distances, seed control, post-harvest monitoring, etc.) are employed? The panel determined that if appropriate confinement is maintained according to established international protocols⁴⁵ the risk from gene flow associated with a CFT is negligible.

Question 5: If assessment indicates that the environmental risk associated with the commercial release of Bt-cowpea is low, with an acceptable degree of uncertainty, what might be the value of implementing a post-commercialization program to monitor changes in wild and weedy populations and how might this monitoring be accomplished? If low environmental risk is indicated by a pre-approval risk assessment the panel determined that monitoring for consequences of gene flow post-approval should not be necessary. Additional ERA studies should be conducted when uncertainties are so large that post-market monitoring would be required.

Summary—Gene Flow in Cowpea

Following consideration of the events above, the panel discussed which of these hypotheses would be the most informative to test for the purposes of a risk assessment. Insecticide sprays to test the hypothesis in event 4 and seed addition experiments to test the hypothesis in event 5 were both considered by the panel. Both of these experiments were considered to be informative.

The panel concluded that seed addition experiments would be the most informative. These experiments would test the ‘worst case scenario’ that there will be an increase in seed (event 4) due to reduction in predation by susceptible insects (event 3) associated with an increase in insect resistance (event 2) in wild cowpeas with the Bt gene (event 1), even though these events have not all been tested and may not be true.

The precise details of the needed experimental design were not determined by the panel, but some important points were considered. It was suggested that seeds could be collected from wild cowpea and sown at typical densities and increasing densities and monitored for germination and survival to reproduction. All of the plots could be sprayed with an insecticide to eliminate insect predation as a factor, since the increased seed would be produced from insect resistant plants. It will be essential to conduct the experiments in multiple, diverse locations, including locations where the seeds were collected. If no correlation is found between the number of seeds and the number of reproductive adult plants, this would demonstrate low risk that the identified harms will occur. If there are more reproductive adults in the higher density plots, this would indicate a need for additional studies.

To determine whether the identified harms discussed above are likely, the panel concluded that insect surveys (event 2) in wild cowpea would not be necessary. However, as discussed later in this paper, insect surveys in wild cowpea related to the issues of

non-target organism (NTO) impacts and insect resistance management (IRM) are recommended.

Panel Discussion: Potential Impact of Bt-Expressing Cowpea on Non-Target Organisms

Before commercial deployment of an insecticidal GM-crop a risk assessment must be conducted to determine the level of risk to biodiversity in general²¹ and to NTOs in particular. NTOs include primarily non-pest arthropods including threatened and endangered animals.^{16,18,46} This risk assessment for Bt-cowpea will be conducted using internationally recognized approaches.²⁴ As mentioned previously, the assessment begins with a problem formulation phase that outlines protection goals, assessment endpoints and testable risk hypotheses that lead ultimately to a characterization of the risk.⁴⁴ The NTO risk assessment is necessarily conducted for the environment in which the GM crop will be grown. However, the uniformity and harmonization of the process used to assess biotechnology derived crops allows for substantial data transportability, i.e., data from international tests conducted on the transgene and protein of interest, e.g., data on Cry1Ab, can be used in regulatory submissions throughout the world.^{28,30}

Question 1: For NTO exposed to Cry1Ab, do the current safety data and history of safe use of Cry1Ab and closely related Cry proteins, namely Cry1Ac and Cry1A.105, provide the necessary NTO safety data for Bt-cowpea expressing Cry1Ab? The panel determined that the current safety data and history of safe use of Cry1Ab and closely related Cry1 proteins provide the necessary NTO safety data for Bt-cowpea expressing Cry1Ab. Specifically, the panel found that:

(a) *Worldwide, governmental regulatory agencies have evaluated Cry1A containing biotechnology-derived products to determine the potential for direct or indirect toxic effects on non-target organisms including arthropods, birds, mammals and humans.*¹⁶ Non-target arthropods evaluated include: (i) beneficial insects representative of the agricultural environment, (ii) a range of taxa found in and around agricultural fields and (iii) threatened or endangered species found in the US¹⁸ or Europe.⁴⁷

(b) *The safety of Cry1A containing products is based on three important assessments.* These assessments are: (i) an understanding of the mode of action and specificity of Bt Cry toxins; (ii) direct testing in feeding bioassays with NTOs and (iii) the long history of safe use of Bt Cry toxins both as insecticidal sprays⁴⁸ as well as expressed in planta.¹⁶

(c) *Cry1A toxins have been shown to have a very narrow spectrum of activity targeting insects in the order Lepidoptera.* Basic research has established that the specificity of the Cry1A class of Bt insecticidal proteins is dependent in part upon their binding to specific receptors in the insect mid-gut.^{16,49} These Cry receptors are not present in non-target birds, mammals and humans.^{16,50-55}

(d) *Within the Arthropoda, the toxicity and specificity of the lepidopteran specific Cry1A proteins are further associated with their solubilization and proteolytic activation in the insect midgut.* This occurs before binding to specific cell membrane receptors in the

Table 1. Effects tests conducted on non-target organisms in support of registered Bt-containing crops in the US

Test Material and Doses	NTO ¹	Result
Cry 1Ab		
50,000–100,000 ppm cornmeal	Bobwhite quail (Bird)	No treatment adverse effects
100–150mg/mL corn pollen	<i>Daphnia magna</i> (water flea)	No treatment adverse effects
20 ppm Cry1Ab protein	Honey bee adults and larvae	No treatment adverse effects
20 ppm Cry1Ab protein	Ladybird beetle	No treatment adverse effects
20 ppm Cry1Ab protein	Parasitic hymenoptera	No treatment adverse effects
16.7 ppm Cry1Ab protein	Green lacewing	No treatment adverse effects
200 ppm Cry1Ab protein	Collembola	No treatment adverse effects
200 ppm Cry1Ab protein	Earthworms	No treatment adverse effects
Cry1Ac		
100,000 ppm	Bobwhite quail (Bird)	No treatment adverse effects
20 ppm Cry1Ab protein	Honey bee adults and larvae	No treatment adverse effects
20 ppm Cry1Ab protein	Ladybird beetle	No treatment adverse effects
20 ppm Cry1Ab protein	Parasitic hymenoptera	No treatment adverse effects
20 ppm Cry1Ab protein	Green lacewing	No treatment adverse effects
Cry1A.105		
550 ppm Cry1Ab protein	Honey bee adults	No treatment adverse effects
1100 ppm Cry1Ab protein	Honey bee larvae	No treatment adverse effects
240 ppm Cry1Ab protein	Ladybird beetle	No treatment adverse effects
240 ppm Cry1Ab protein	Parasitic hymenoptera	No treatment adverse effects
80 ppm Cry1Ab protein	Collembola	No treatment adverse effects
120 ppm Cry1Ab protein	<i>Orius insidiosus</i>	No observed effect concentration 120 ppm

Sources:^{18,60} ¹Species names of NTOs tested are not provided since the actual species, and in some limited cases the genus, has changed over time. The taxonomy of the tested animals is available through the US EPA website and is specific for each registered biotech product.

brush border membrane present in the midgut of susceptible insects. To date the Cry1A toxins have all been shown to be specific for lepidopteran insects. Although some have suggested that insects in closely related taxa such as the Trichoptera⁵⁶ might also be susceptible, careful evaluation reveals that this appears not to be the case.⁵⁷⁻⁵⁹

(e) *Data demonstrating safety for non-Lepidoptera species for all commercialized Cry1A class toxins are extensive.*^{16,18,60,61} All commercialized Bt Cry toxins have been extensively tested in the laboratory against a wide range of arthropods typically at concentrations at least 10X the expected environmental concentration (EEC)^{16,18} See also the US EPA Biopesticide Registration Action Documents in Table 1. In addition, extensive testing in several academic laboratories supports the safety of Cry1A Bt toxins to natural enemies^{16,62,63} and honey bees.⁶⁴ Substantial data sets from field studies have also been the subject of extensive Meta analysis at the taxonomic⁶⁵ and functional guild level;⁶⁶ they support the conclusion of safety.¹⁶

Question 2: Does the expression profile of Cry1Ab in cowpea effectively remove some NTO groups from consideration? The expression profile of Cry1Ab in cowpea effectively removes some NTO groups from consideration. However, current data on the expression profile of Cry1Ab in Bt-cowpea is limited because the project is in the event selection phase. The first events evaluated suggest that expression levels of Cry1Ab in pollen are near the limit of detection (approaching zero). If this

finding is substantiated then arthropods, e.g., bees, which are potentially exposed to Cry1Ab through pollen could be excluded from consideration due to lack of exposure. Similarly, seed-feeding organisms may be removed from consideration if Cry1Ab is not expressed (or only at low levels) in Bt-cowpea seeds. Based on experience with other Cry1A-expressing crops, it is likely that the protein will not be transported in the plant's phloem sap and thus not ingested by aphids.⁶⁷ Consequently, the risk to natural enemies that exclusively or predominantly feed on aphids can be assumed to be negligible because of minimal exposure.

Question 3: Are there species that occur in Africa and in cowpea (considering exposure) for which additional testing would be necessary? There do not appear to be any unique species (taxa) in the African receiving environment for which additional testing of their sensitivity to Cry1Ab would be warranted because they belong to higher taxa that are adequately covered already. However, consultation with regional regulators will determine whether the available non-target data on Cry1Ab together with field surveys of the arthropod fauna (see question 5) in Bt-cowpea fields in the region will be sufficient for a regulatory assessment.

However, since gene flow is expected to occur to wild cowpea plants, NTOs might be exposed to the insecticidal trait outside the cowpea crop, when feeding on wild relatives that express Cry1Ab. Thus additional testing might be required to address the risk to these organisms if they are unique to wild cowpea

Table 2. Parasitoids and predators associated with the common cowpea insect pests *Maruca vitrata*, *Megalurothrips sjostedti* and *Clavigralla tomentosicollis* in West Africa

Host	Parasitoid/Predator Order	Family	Genus/Species	Reference
<i>Maruca vitrata</i>	Hymenoptera	Trichogrammatidae	<i>Trichogrammatoidea eldanae</i>	Arodokoun et al., 2006
<i>Maruca vitrata</i>	Hymenoptera	Braconidae	<i>Phanerotoma leucobasis</i>	Arodokoun et al., 2006
<i>Maruca vitrata</i>	Hymenoptera	Braconidae	<i>Apanteles taragamae</i>	Srinivasan et al., 2007
<i>Maruca vitrata</i>	Hymenoptera	Braconidae	<i>Braunsia kriegeri</i>	Arodokoun et al., 2006
<i>Maruca vitrata</i>	Hymenoptera	Braconidae	<i>Pristomeru</i> spp	Arodokoun et al., 2006
<i>Maruca vitrata</i>	Hymenoptera	Braconidae	Bracon spp	Arodokoun et al., 2006
<i>Maruca vitrata</i>	Hymenoptera	Braconidae	Dolichogenidea spp	Arodokoun et al., 2006
<i>Maruca vitrata</i>	Hymenoptera	Braconidae	Testudobracon spp	Arodokoun et al., 2006
<i>Megalurothrips sjostedti</i>	Hymenoptera	Trichogrammatidae	Megaphragma spp	Tamò et al., 1993
<i>Megalurothrips sjostedti</i>	Hymenoptera	Trichogrammatidae	Oligosita spp	Tamò et al., 1993
<i>Megalurothrips sjostedti</i>	Hymenoptera	Eulophidae	<i>Ceraninus menes</i>	Tamò et al., 1993
<i>Megalurothrips sjostedti</i>	Hymenoptera	Eulophidae	<i>Ceraninus femoratus</i>	Tamò et al., 2003
<i>Clavigralla tomentosicollis</i>	Hymenoptera	Scelionidae	<i>Gryon fulviventris</i>	Asante et al., 2000
<i>Clavigralla tomentosicollis</i>	Hymenoptera	Encyrtidae	<i>Ooencyrtusutethesia</i>	Asante et al., 2000
<i>Clavigralla tomentosicollis</i>	Hymenoptera	Eupelmidae	Anastatus spp	Asante et al., 2000

Tamò, M, Baumgärtner J, Delucchi V, Herren HR. Assessment of key factors responsible for the pest status of the bean flower thrips *Megalurothrips sjostedti* (Thysanoptera: Thripidae) in West Africa. *Bull Entomol Res* 1993; 83:251-8.

Arodokoun, DY, Tamò M, Cloutier C, Brodeur J. Larval parasitoids occurring on *Maruca vitrata* Fabricius (Lepidoptera: Pyralidae) in Benin, West Africa. *Agriculture, Ecosystems & Environment* 2006; 113:320-5.

Tamò M, Ekesi S, Maniania N, A C. Biological control, a non-obvious component of integrated pest management for cowpea. In: Neuenschwander P, Borgemeister C, J L, eds. *Biological control in integrated pest management systems in Africa*. Wallingford, UK: CABI Publishing, 2003:295-309.

Srinivasan R, Tamò M, Ooi P, Easdown W. IPM for *Maruca vitrata* on food legumes in Asia and Africa. *Biocontrol News and Information* 2007:34N-7N.

Asante, S, Jackai L, Tamo M. Efficiency of *Gryon fulviventris* (Hymenoptera: Scelionidae) as an Egg Parasitoid of *Clavigralla tomentosicollis* (Hemiptera: Coreidae) in Northern Nigeria. *Environ Entomol* 2000; 29:815-21.

and they feed in a manner that would expose them to the toxin. Currently the knowledge of the arthropods living on wild cowpeas in Africa is poor and has to be addressed with additional research.

Question 4: Does the fact that cowpea is primarily a self-pollinating crop remove the need to consider exposure issues associated with pollen? Even in the event that Bt-cowpea pollen contained substantial levels of Cry1Ab (which is probably not the case, see **question 2**) it is not dispersed and consequently lepidopteran larvae are unlikely to be exposed. The only organisms that could potentially ingest Cry1Ab contained in pollen are bees. There is, however, strong evidence that Cry1Ab does not affect Hymenoptera in general or honey bees,⁶⁴ bumble bees⁶⁸ or solitary bees⁶⁹ in particular. Based on these toxicity data the need to test additional Hymenoptera is not warranted.

Question 5: What data are needed to establish familiarity regarding non-target insects in Bt-cowpea compared with non-Bt-cowpea? Familiarity data are data collected to establish the level of similarity in ecologically relevant characteristics between the GM crop and its non-transformed comparator.¹⁹ Typically in the US, where the vast majority of GM crops have been evaluated, familiarity data are collected as part of the regulatory agronomic assessment which considers a small number of non-target pest and beneficial species. In some African countries these data will be part of a larger field survey. It is anticipated that the familiarity data typically collected for GM crops will also be collected for Bt-cowpea.^{16,18,46}

The efficacy data that will be collected include effects on target and non-target arthropods (primarily insects). In addition, as part of the regulatory product field evaluation, selected and focused regulatory assessments are made on each of these subject areas including information on target and non-target pest and beneficial insects. These data might include selections from NTOs known to occupy the cowpea agroecosystem (Table 2). Together these data provide a strong weight of evidence argument in support of the familiarity conclusion of safety in the dossier.⁶¹

Question 6: Are there endangered or threatened species that need to be considered? Endangered and threatened species are routinely addressed in virtually all environmental regulatory submissions. Many countries lack a comprehensive endangered and threatened species database. Instead, they rely on the IUCN Red List of Threatened Species™. The listing is organized by country and an assessment will need to be made on a country by country basis. The expert panel reviewed a draft Red list of endangered insects for Benin, a country in West Africa near Nigeria. None of the 30 proposed species on that list occur on legume plants and the likelihood that an insect control measure on cowpea would impact them is low (Georg Goergen, IITA, pers. comm.).

Summary—Potential Impact of Bt-Expressing Cowpea on Non-Target Organisms

Before commercial deployment of Bt-cowpea in West Africa a risk assessment will be conducted to determine the level of risk

to NTOs, including threatened and endangered species that are exposed to the crop. This NTO risk assessment will be conducted using internationally recognized approaches.^{16,18,19,24} The first step of that process is the problem formulation phase, which is informed by expert opinion and regulatory policy and is presented here. The expert panel addressed six specific questions associated with the potential environmental risk of Bt-cowpea to NTOs. The panel determined that for NTOs exposed to Cry1Ab in cowpea the current safety data and history of safe use of Cry1Ab and closely related Cry proteins, namely Cry1Ac and Cry1A.105, provide important NTO safety data for Bt-cowpea expressing Cry1Ab. This assessment is based on the assumption that the concentration of Cry1Ab in Bt-cowpea will fall in the range of previous assessments and the taxonomic breadth of the ecotoxicology data adequately predicts the effects of Cry1Ab to NTOs potentially exposed to the protein via Bt-cowpea in the field in West Africa. Further the currently known expression profile of Cry1Ab in cowpea effectively removes some NTO groups from consideration, e.g., the natural enemies of phloem-feeding aphids, since these organisms would have limited or no exposure to Cry1Ab. Currently, there do not appear to be any NTO species that occur in Africa and in cowpea (considering exposure) for which additional testing would be necessary. However, because of the likelihood of gene flow to wild cowpea the panel recommended further assessment of published papers and institutional reports as well as field survey work to determine whether any unique species might be exposed in wild cowpea. The panel also outlined—based on current international guidelines—the likely data needed to support the familiarity component of the registration package. These data would include in field assessments of select NTOs collected in the product development and regulatory registration phases. Finally there do not appear to be any threatened or endangered species associated with cowpea in West Africa. In summary, the key data needs for this phase of the risk assessment are primarily associated with target and non-target organisms, primarily arthropods, associated with wild cowpeas growing in the areas where Bt-cowpea will be deployed.

Panel Discussion: Potential for Target Pest Populations to Evolve Resistance to Cry1Ab Protein

Insect Resistance Management (IRM) is a key component to the sustainable use of all insecticides including those used in planta via GM crops.³¹ It must be noted that IRM is not a safety issue but rather a component of product stewardship that seeks to maximize the duration of resistance genes in deployed transgenic crops. The expert panel considered the possible evolution of LPB populations resistant to the Cry1Ab protein as a first step in developing appropriate IRM plans for Bt-cowpea in West Africa. An important aspect of those deliberations was the clear recognition that existing IRM approaches as applied to Bt crops in other regions of the world serve as a useful backdrop for the case of LPB in West Africa. The panel concluded that one can develop a viable, robust IRM plan for Bt-cowpea in West Africa but there are areas where clarification of the existing knowledge base will be needed to design the most appropriate approach. The

discussion and answers to nine questions posed to the panel of experts are summarized here and provide the basis for developing IRM plans in countries of West Africa where Bt-cowpea will be made available to farmers.

Question 1: On a regional basis in areas where Bt-cowpea will likely be sown in substantial hectareage, what is the distribution and abundance of alternative hosts of *Maruca vitrata* that could support sufficient susceptible populations to provide mating partners for *M. vitrata* surviving in Bt-cowpea fields? Discussion centered on those countries in West Africa with the largest cowpea production and where Bt-cowpea will likely be initially released: Nigeria, Burkina Faso and Ghana. The outcomes, however, are generally applicable to other countries in West Africa where Bt-cowpea may eventually be grown: Niger, Mali, Benin and Togo. Although the conclusions are generally applicable to many areas of the African continent, further specific information may be required especially for northern Burkina Faso and Niger. Areas of cowpea production in East Africa, which are not currently under consideration for Bt-cowpea introduction, represent a markedly different situation and were not considered.

West Africa has three major zones relevant to LPB and its host plants. These are the Southern coastal forests; the central savannah which transitions from the wetter, semi-wooded Guinea zone to a classical grassy savannah; and in the north, the Sahelian zone, consisting of dry scrub with occasional grasses and patches of bare earth. Domestic cowpea production occurs sporadically in the south, is most intense throughout the savannah, and extends to the Sahelian zone.⁷⁰ Alternative host plants for LPB predation occur throughout West Africa but their distribution and relevance as refugia for Cry1Ab-susceptible LPB populations will be dependent on the particular zone considered.

Evidence thus far supports the hypothesis that LPB shows an annual cycle of south to north movement following the rainfall pattern and availability of host plants. LPB is endemic to the Southern forest zone where ample host plants exist year round, primarily as leguminous trees. These trees are the more consequential host for LPB in this zone where there is no extensive production of domestic cowpea. Throughout wetland savannah areas and particularly in the Guinea savannah, *Sesbania* appears to be a significant host for LPB. However, its restricted localization means that its natural populations cannot be viewed as a dominant alternate host for LPB everywhere within this zone. Wild cowpea (*Vigna unguiculata* ssp. *spontanea*), and perhaps surviving volunteer domesticated cowpea may occur throughout the savannah but their distribution and density varies considerably. Wild cowpea becomes increasingly less common in transitioning from the Guinea zone to grassy savannah in the northern reaches of the central zone. LPB in the central zone are largely non-endemic, but some localized endemic populations may exist within the Guinea zone largely in moist areas such as along rivers (M. Tamu, personal comm.). Up to seven generations of LPB may occur annually in this zone. LPB occurring within the Sahelian zone are non-endemic and go through annual extinction. Like other zones it is possible for small LPB populations to exist endemically along moist areas such as rivers (M. Tamo, personal comm.). However, in Southern Burkina Faso, an area

where cowpea is grown,⁷¹ *Maruca* was observed on wild alternative hosts throughout the year.

The cycle of south to north movement through the region of cowpea production, with annual influx of susceptible LPB from the south and annual extinction of potential recessives in the north, acts as a potentially strong natural mechanism to limit the evolution of Cry1Ab-resistant populations. The significance of endemic populations in the Guinea zone and clarification of the inter-mating of these with the northward migration of LPB will be needed. In addition, the relative importance and prevalence of LPB hosts within the central zone requires further definition to assess their relevance as potential refugia for Cry1Ab-susceptible LPB. In particular, there will need to be surveys to determine whether wild cowpea represents a significant alternate host for LPB, providing a population of non-selected mating partners. Surveys should determine the degree of predation of wild cowpea by LPB as well as the distribution and density of wild cowpea relative to domestic cowpea production.

Question 2: What is known about the short and long-distance movement (flight) behavior of *Maruca vitrata* populations? How might this behavior serve to hasten or delay resistance development in *Maruca vitrata* populations? There are ample observations to substantiate that LPB mating occurs outside of cowpea fields and that inter-mating will occur among LPB coming from differing host plants.^{72,73} Thus, inter-mating of resistant LPB emerging from cowpea fields with susceptible LPB from refugia can be a facet of resistance management. However, the spatial and temporal aspects of the refugia in relationship to Bt-cowpea cowpea fields will be further defined when the final Bt-cowpea event is chosen and evaluated for deployment.

Long-distance movement (that is, south to north migration) of LPB occurs in response to food source availability. It has been hypothesized that there is negligible north to south movement.^{71,74} The pattern of long distance movement in conjunction with lack of diapause and extinction within the Sahelian zone will act to delay resistance development provided endemic populations are either absent from the Guinea zone or they inter-mate with transients.

Question 3: What is the likelihood that *M. vitrata* larvae that survive exposure to Bt-cowpea, but are developmentally delayed, will be able to complete their development and pass on their genes to the next generation? For long-term durability of Bt-cowpea, the expression of the Cry1Ab protein in cowpea tissue on which LPB feeds needs to be sufficient to assure LPB cannot complete their life cycle. If this is accomplished, the ability for larvae to survive, complete development and pass genes to the next generation will be impacted by larval movement within and among cowpea plants. LPB first instar larvae feed on flowers and then move to green tissues (primarily the developing pods) as second or later instars. If an intoxicated larva moves from a flower to a pod there must be sufficient levels of Cry1Ab in the green tissue to assure continued exposure to a lethal dose of the protein. Data on the sensitivity of different larval stages toward Cry1Ab will be of benefit when considering on-plant movement.

The potential for movement from Bt-expressing cowpea plants to adjacent non-Bt-cowpea plants was considered. The development plan for Bt-cowpea in West Africa takes into account the tradition of “plant-back” of saved seed. To what extent Cry1Ab expression levels remain consistent in these plant-back seed populations would be useful data to obtain. It is anticipated that new seed will be purchased on a three-year cycle since discussions with seed sellers suggests that most farmers in the region replace their seed about every three years.

Cultivated cowpea is overwhelmingly (~99%) self-pollinating. As it is anticipated that farmer-deployed Bt-cowpea lines will be homozygous for the Bt trait, this will essentially neutralize the opportunity for trait segregation within the Bt-cowpea field. Since cowpea varieties are inbred lines,⁷⁵ and the Bt construct therefore fixed in the variety, farmer saved seed will continue to breed true for the Bt resistance gene for many generations. Rare outcrosses to non-Bt-cowpea will produce a heterozygous F1 that will segregate for resistance in the F2 and subsequent generations. However, because outcrossing is rare (often <1%), these F1s and subsequent susceptible F2 will be rare, the opportunity for trait segregation is minimal. Furthermore, outcross F1 individuals are easily recognizable in seed production fields as large off-type plants which can be removed to help maintain seed stock purity.

Some outcrossing occurs between cultivated and wild-weedy species⁹ and a question to consider is whether pollen from these dispersed low-density wild/weedy populations moves at any substantial frequency into cultivated plantings of cowpea. From a source-sink perspective, one might conclude that the likelihood of this occurrence to be very low, but this issue can be examined by PCR analysis of border row plants from field plots of cultivated cowpea growing in areas where wild/weedy plants are also observed. That the likely occurrence of wild gene flow into cultivated Bt-cowpea will be low is supported by the observation that flowers of wild/weedy cowpea tend to open and close much earlier in the day than cultivated lines.⁷⁶ As it has also been reported in reference 76, that bees (pollen vectors) typically revisit their hive prior to visiting flowers of cultivated cowpea (whose flowers open later in the day), this would further reduce the likelihood that non-Bt pollen from a wild/weedy plant would successfully pollinate a cultivated line, and further diminish the likelihood that the Bt gene would be diluted in the cultivated plantings. A combination of studies and/or resistance modeling at the plant-to-plant scale will be helpful to fully evaluate the significance of plant mixtures, but only if introgression of non-Bt pollen into the cultivated Bt-cowpea field is determined to occur at a meaningful level.

Question 4: Is the 25X LC99 standard as applied to crops such as Bt corn and Bt cotton in the United States a critically important standard for the selection of a Bt-cowpea event? The 25X LC99 standard was originally adopted in the United States for maize and cotton to address uncertainties in USEPA's earliest IRM plans regarding the allele frequency governing emergence of resistant insects.⁷⁷ Briefly, this standard specifies that the concentration of the toxin (e.g., Cry1Ab) expressed in planta should be at a level that is 25 times the lethal concentration (LC) needed to kill 99% of the target test species in an artificial bioassay.

This conservatively-cast projection has been used as the upper bound for addressing uncertainty but is not necessarily relevant on the basis of current knowledge regarding resistance models and the management of resistance. It appears especially arbitrary for the case of Bt-cowpea in West Africa. Further, this projection was based upon an agricultural system in the United States where very dense plantings of Bt-expressing crops over a broad land area were anticipated, and where natural refugia were limited or absent. If further consideration of presence and or mating behavior of endemic LPB populations in the Guinea zone shows little evidence for evolution of locally resistant populations, then expression levels do not need to be significantly higher than those needed for product efficacy (because of the annual extinction of populations migrating into cowpea production areas). As new data and further observations emerge, this topic can be re-visited to determine if additional measurements would be beneficial to support a sustainable resistance management plan for Bt-cowpea. For instance, resistance management plans may benefit from estimates of allele frequency developed from measurements or modeling as well as susceptibility and variance estimates for LPB which confirm data from other regions.⁷⁸ Alternatively, if at least two non-competitive Bt proteins (e.g., Cry1Ab and Cry2Ab2) are expressed in planta, the 25X LC99 standard is less important and risk management will be simplified. Even in situations where the 25X LC99 standard is not met the event may still be commercialized. This was the case for corn rootworm resistant MON 863 maize, which expressed a single *cry3* gene whose toxin effects were significantly less than the 25X LC99.⁴¹

Question 5: Would a seed mixture of a Bt-cowpea and non-Bt-cowpea be a viable management option for thwarting resistance development in Bt-cowpea? Would a deployment program of this nature be sustainable? This question is not easily answered given the currently available knowledge described in **question 3** above. However, if there is a data-supported concern for plant mixtures in fields as a challenge to resistance management in this region (see **question 3**), it is unlikely a seed mixture will be an effective refuge option. The specific implications of seed mixtures may be best addressed through modeling.

Question 6: Is a structured refuge, by which individual farmers will be required to plant a certain percentage of their cowpea hectare to non-Bt cultivars or other alternative hosts to sustain un-selected populations of *M. vitrata*, a viable management option for Bt-cowpea deployment in Africa? Two key unknowns exist in devising the IRM strategy for Bt-cowpea in West Africa. These are (1) the significance of endemic LPB populations in the Guinea zone as well as clarification of the inter-mating of these with northward migrating LPB and (2) determination as to whether wild cowpea is a significant alternate host for LPB in the Guinea zone (**question 1**). In view of these unknowns, the present worst case assumption must be that wild cowpea does not represent an alternate host for LPB.

A structured refuge coupled with high-dose production of toxin in Bt-cowpea is a viable IRM strategy. There are concerns however, that farmer's may not plant the refuge as required. Reports from South Africa suggest that farmer compliance is poor for Bt corn hybrids.⁷⁹⁻⁸¹ However, with ample infrastructure

for grower education and follow-up, a structured refuge approach could be envisioned for West Africa and cowpea. Alternatively, other refuge strategies were considered: structured native refuge (such as establishing border areas of *Sesbania* or other leguminous trees); an alternate host crop such as pigeon pea; community refugia; or structured refugia for large commercial operations only. The possibility for any of these options is best investigated through the use of IRM models that allow for the various scenarios to be tested.

Question 7: In terms of resistance development, are there other lepidopteran pests of cowpea, in addition to *M. vitrata*, that should be considered? No other lepidopteran pests occur in sufficient quantities in cowpea to warrant consideration.

Question 8: How would a transgenic cowpea line expressing multiple lepidopteran-active insecticidal ingredients change the IRM requirements? Expression of more than one lepidopteran active protein with a different mode of action (such as in a pyramided product) can potentially reduce the size of the refuge needed for a durable IRM plan.⁸² Thus, a second protein effective against LPB (such as Cry2A) needs to be considered as part of the long-term strategy for durable LPB control in cowpea. Introduction and implementation of a second gene could either be in tandem with Cry1Ab on a single construct (preferred), or the second gene could be introduced at a later date if necessary. The single construct option would have the advantage of simplifying any subsequent breeding to introgress the genes into new cultivars and would be of benefit for those farmers who save seed from year to year. If two toxins are not derived from a single construct (i.e., the toxin genes assort independently) then seeds saved by farmers for two or more years would produce plants with zero, one or two toxins, which could present a challenge to a durable IRM strategy. This issue can be addressed as follows.

Considering farmer-grown seed production in Africa, growing Bt-cowpea in proximity to non-Bt cowpea over a considerable number of generations, combined with a moderate to high level of outcrossing (>1%) could result in the presence of a significant number of single event (i.e., Cry1Ab or Cry2A) individuals being present in a Bt variety over time. These individuals would present greater opportunities for evolution of resistant forms (that could arise by matings of complementary forms or an additional mutation for resistance) and represent a minor challenge to a durable an IRM strategy. However, simple seed production practices can be put in place with farmers involving removal of off-type F1 plants in seed production fields (the F1s that were formed the year before from outcrosses are generally easily recognized by size and morphology). If some F1 plants escape the removal process, removal of off-type seeds (the F2 seeds from these F1 outcrosses) can be practiced easily as these generally do not look like the seed of the variety in harvested seed lots. These practices will minimize the opportunity of single event individuals arising. To further reduce this problem, farmers should be instructed to produce seed from known pure seed stocks every few years.

Question 9: What is the panel's views and vision for a monitoring program if Bt-cowpea were to be deployed? A monitoring program is an important consideration for life cycle stewardship of Bt-cowpea in West Africa. It is anticipated that a Bt-cowpea

product will lead to considerable increases in cowpea production and cowpea will become an even more critical component of West African food production systems. This further emphasizes the urgency of designing and deploying a robust IRM program that fits in well with local farmer practices. The monitoring program to be developed should depend on existing infrastructure such as extension agents and seed representatives as the monitoring focus. Field surveillance of product performance and investigating reports of unexpected levels of insect feeding damage can be two focal points for the monitoring effort.

Summary—Potential for Target Pest Populations to Evolve Resistance to Cry1Ab Protein

Development and sustainable deployment of Bt-cowpea for West Africa requires effective resistance management. Choosing IRM practices that can readily be adopted by African farmers without radically disrupting local cultural tendencies is crucial for success of the program. Deploying a Bt-cowpea (preferably with two insecticidal proteins) that expresses a sufficiently high level of insecticidal protein to kill all exposed larvae is a critical factor to prevent development of resistant populations. Additionally, unique attributes of LPB biology in West Africa afford opportunities for resistance management. In the Southern forest zone, where cowpea is less extensively cultivated, there are alternate hosts and endemic LPB while the northern zone has scarcity of hosts and LPB appear to die out seasonally. South to north migration patterns with negligible north to south back migration mean that endemic LPB from the south can provide susceptible moths in the savannah zones where LPB is non-endemic and goes extinct in the northern-most cowpea-growing zone. With adequate alternate hosts, mating outside of cowpea fields and mating among endemic and transient populations, there is potential for a natural mechanism to control the evolution of resistant populations of LPB. Further studies are important to supply the quantitative information needed for use with appropriate simulation models.

The alternate host distribution and abundance in the central zone is not well understood and this information is needed to ensure the appropriate ratio of susceptible to resistant LPB for resistance management. Wild cowpea may be the most relevant alternate host in this zone, but in addition to uncertainties regarding its distribution and density relative to cowpea fields, there is uncertainty as to the extent of LPB host choices and the consequences should wild cowpea refugia be impacted by gene flow from Bt-cowpea.

Because alternate hosts may be limiting or uncertain, use of structured refugia may be necessary if adoption of Bt-cowpea becomes very concentrated. The nature of these refugia will depend on the Cry1Ab expression level and pattern as well as local farming practices. Because of the communal nature of subsistence farming in Africa communal refugia should also be considered.

Information generated from ecological patch (mosaic) models for resistance management have proven useful in developing IRM programs for other Bt crops grown outside of Africa. These models may also prove useful for addressing uncertainties for Bt-cowpea-cowpea in West Africa. Models of plant-to-plant

(in-field) movement can address the impact of mixed stands of Bt and non-Bt-cowpea in the same field (if they exist). Landscape scale models may address distribution and density of alternate hosts to ensure that appropriate ratios of susceptible to resistant insects are maintained, and regional scale models may be used to confirm the effectiveness of south to north migration in elimination of resistant LPB. Required for the use of models are reliable estimates of allele frequencies and susceptibilities for LPB.

Recommended Data—Gathering Activities Based upon the Deliberations of the Expert Panel

Gene flow. First priority. Test the hypothesis that an increase in seed production by wild/weedy cowpea (*Vigna unguiculata* ssp *unguiculata* var *spontanea*) does not result in an increase in abundance of wild cowpea. This may be done by collecting wild cowpea seeds and sowing them at different densities and subsequently assessing whether initial seed density affects the resulting plant stand. Plant vigor as well as density should be determined. These tests should be conducted at multiple locations—at least three or more, preferably at sites representing different ecologies and including localities where Bt-cowpea may eventually be deployed (i.e., at least one site in Nigeria, one in Burkina Faso and one in Ghana). Plant stands should be followed for at least two years or perhaps longer if the first two years of observations point toward persistent effects on plant stand vigor or density.

Second priority. Test the hypothesis that insect infestation reduces survival or seed production of wild/weedy cowpea. This can be done by spraying stands of *V. u.* ssp *u.* var *spontanea* with a broad-spectrum insecticide that kills lepidopteran pests. This is a worst-case scenario, however, since all insects will be affected, not just Bt-sensitive ones. A specific test for the effect of lepidopteran insects on cowpea fitness could be made using a commercial lepidopteran-specific Bt preparation as well. Sprays should be repeated at regular and frequent intervals to ensure maximal control, with control plants being sprayed with the carrier without insecticide. Subsequent evaluations should examine the plants for measures of vigor, growth, flower production, presence/absence of insects and above all, seed yield and viability. Tests should be conducted at three independent sites with statistically adequate replications at each site.

Third priority. Test the hypothesis that lepidopteran insects controlled by the Cry1Ab protein infest wild cowpea. Regular observations of natural stands of wild cowpea should be performed over the course of the cowpea growing season to describe and document the insects associated with the plants. Nearby cultivated cowpeas should be monitored as a basis for comparison. Given the lepidopteran specificity of Cry1Ab, particular attention should be given to lepidoptera associated with the wild/weedy plants. Because insect populations fluctuate in size and species composition from year to year and vary across their geographical ranges, observations of the insect fauna associated with wild cowpea should be done in at least three different sites, e.g., particularly in Burkina Faso and Nigeria. Wild/weedy cowpea populations in Ghana are limited to the extreme north of the country (Pasquet R, unpublished observation).

Non-target organisms. *First priority.* It is desirable to have more detailed knowledge of the non-target insects associated with wild/weedy cowpea, particularly lepidopteran insects. This reinforces the need for research on insects and other organisms associated with wild/weedy cowpea described in the Third Priority above.

Second priority. Compile information and synthesize this into a report that will support and advance our understanding of non-target organisms of Bt-cowpea (similar to the study conducted earlier in ref. 30). Sources of information include not only the published literature but annual reports of such organizations as the National Agricultural Research Programs, IITA, and the Bean/Cowpea CRSP. This work should be performed by a consultant who is already knowledgeable about LPB biology and who can get access to the relevant reports comparatively easily.

Insect resistance management. *First priority.* Determine the concentration of Cry1Ab protein in the tissues of cultivated cowpea, *Vigna unguiculata* ssp *unguiculata*. The primary focus should be on those tissues targeting LPB, in particular flowers parts and pods. The assessment should also consider the likely growth stage(s) infested by LPB. Other tissues should be assessed, including roots, leaves, male and female reproductive tissues, pollen and stems.

Second priority. Carry out an assessment of (1) the distribution and abundance of alternative hosts of *M. vitrata* and (2) the extent to which farmers in likely Bt-cowpea recipient countries grow local cowpea varieties for home consumption; such cowpeas might serve as refuges.⁸³ This needs to be a region-wide study covering the area where Bt-cowpea is likely to be disseminated.

Conclusion

Africa needs more, cheaper and safer food produced with a minimum of inputs. Farmers need the cash incomes increased food crop production brings. Consumers need an ever-growing supply of food they can buy at reasonable and stable prices. In the years ahead, as the world population adds two billion or more people, today's food supply, already inadequate in Africa, will become still more inadequate.

In hope of increasing the availability of cowpea, a key African food, scientists from around the world are well on their way to completing development of a Bt-cowpea variety that (i) will increase the supply of that key food, (ii) resist the legume pod borer, a devastating insect pest (iii) increase yields while reducing or eliminating insecticide contamination of food, soil and water (iv) is safe to eat (v) is safe for growers, the environment and consumers (vi) is accepted by African farmers as well as consumers.

More than 20 experts convened at the Danforth Foundation in St. Louis, MO USA, in February 2009 to (i) to identify any real or apparent risks associated with possible future deployment of Bt-cowpea in the cowpea growing region of West Africa (ii) assess those risks critically and objectively (iii) identify information gaps than needed to be filled to complete the safety assessment and (iv) prioritize research to fill information needs—prioritize because funds for this orphan crop are severely limited.

The state of the Bt-cowpea art was carefully analyzed during the expert deliberations; and the results presented here. The sum and essence of the Expert Panel's deliberations are that Bt-cowpea has a promising future. Knowledge from over 25 years experience of creating, selecting and breeding transgenic crops indicates that genetic modification is no more likely to have harmful unintended effects than are other methods of introducing genetic variation into crops, such as wide hybridization and mutagenesis.^{84,85} Consequently, safety concerns about Bt cowpea should focus on the intended transgene and product, not on the method used to introduce it into the crop. Cry1Ab and similar proteins, have been used extensively in transgenic crops worldwide, providing benefits to farmers, consumers and the environment in developed and developing countries.^{14,28,86} The environmental and health risks from crops producing Cry1Ab are well characterized and negligible^{18,62} and the data from which those conclusions are drawn are, in general, applicable to Bt cowpea; therefore, a preliminary assessment of Bt cowpea is that it too poses negligible risk.

Risk assessment studies introduce opportunity costs and may delay the introduction of beneficial products; therefore requests for additional regulatory data are not free from risk. A balance must be struck between the costs of too much testing of activities that pose low risk with the costs of too little testing that fails to reveal activities posing high risk. Hence, proposals for additional testing of Bt cowpea should be examined critically to determine whether their value outweighs their costs. The need for the studies cannot be determined solely by scientific analysis, but will be a judgment by local regulators based on their priorities, which may differ among countries. Ultimately, decisions to require further studies may be made for reasons of risk communication, not because of unacceptable scientific uncertainty about the likelihood of harmful effects of cultivating and consuming Bt cowpea. Acceptability of Bt cowpea will depend on the perception of the risks it poses and studies performed in Africa on Bt cowpea may be more convincing than a weight of evidence from other Bt crops grown elsewhere. The scientific analysis presented here is therefore only part of the evidence on which regulatory data requirements for Bt-cowpea should be based.

In the end, as is proper, the African people themselves will determine whether the benefits of Bt-cowpea and the increased safe food supply it promises outweigh any risks attendant upon deployment of a genetically-modified insect-resistant cowpea. Needed further work, identified here, promises to create an even more thoroughly grounded foundation for the adoption of a Bt-cowpea product.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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