Journal of Pest Science
https://doi.org/10.1007/s10340-018-0974-0

REVIEW

An assessment of the risk of Bt-cowpea to non-target organisms in West Africa

Malick N. Ba[1] · Joseph E. Huesing[2] · Manuele Tamò[3] · Thomas J. V. Higgins[4] · Barry R. Pittendrigh[5] · Larry L. Murdock[6]

Received: 21 April 2017 / Revised: 15 March 2018 / Accepted: 16 March 2018

Abstract
Cowpea (*Vigna unguiculata* Walp.) is the most economically important legume crop in arid regions of sub-Saharan Africa. Cowpea is grown primarily by subsistence farmers who consume the leaves, pods and grain on farm or sell grain in local markets. Processed cowpea foods such as *akara* (a deep-fat fried fritter) are popular in the rapidly expanding urban areas. Demand far exceeds production due, in part, to a variety of insect pests including, in particular, the lepidopteran legume pod borer (LPB) *Maruca vitrata*. Genetically engineered Bt-cowpea, based on *cry1Ab* (Event 709) and *cry2Ab* transgenes, is being developed for use in sub-Saharan Africa to address losses from the LBP. Before environmental release of transgenic cowpeas, the Bt Cry proteins they express need to be assessed for potential effects on non-target organisms, particularly arthropods. Presented here is an assessment of the potential effects of those Cry proteins expressed in cowpea for control of LPB. Based on the history of safe use of Bt proteins, as well as the fauna associated with cultivated and wild cowpea in sub-Saharan Africa results indicate negligible effects on non-target organisms.

Keywords Cowpea · *Maruca vitrata* · Bt-cowpea · Non-target organisms · West Africa · Environmental risk · Assessment · Arthropod fauna

Key messages

- Data on the environmental safety of *Bacillus thuringiensis* (*Bt*) Cry proteins, especially toward non-target arthropods, are available for many genetically modified crops but not in the contest of cowpea.
- Before environmental release of Bt-cowpea for control of the legume pod borer, a major insect pest attacking cowpea, the potential effects on non-target organisms (NTO), particularly arthropods, need to be assessed.
- An assessment of the *Bt* Cry proteins, their history of safe use, as well as the fauna associated with cultivated and wild cowpea in sub-Saharan Africa indicates negligible effects on non-target organisms.

Introduction

Cowpea (*Vigna unguiculata* Walp.) is the most economically important legume crop in West Africa (Langyintuo et al. 2003). Nigeria, Niger and nations surrounding these two countries are the most productive cowpea lands globally,

Communicated by N. Desneux.

✉ Barry R. Pittendrigh
pittendr@msu.edu

1 International Crops Research Institute for the Semi-Arid Tropics, Niamey, Niger

2 United States Agency for International Development (USAID), Washington, DC, USA

3 International Institute of Tropical Agriculture, Cotonou, Benin

4 Commonwealth Scientific and Industrial Research Organisation (CSIRO), Agriculture and Food, Canberra, Australia

5 Department of Entomology, Michigan State University, East Lansing, MI, USA

6 Department of Entomology, Purdue University, West Lafayette, IN, USA

Published online: 10 April 2018

and Africa accounts for over 95% of the world production (FAOSTAT 2017). In much of West Africa, cowpea grains constitute the major source of protein and for this reason are regarded widely as “poor man’s meat” especially in remote rural areas (Murdock et al. 2008). In fact, most of the cowpea grain is consumed at home or sold in local markets. Some of it is traded into neighboring countries to the south, which it reaches following an ancient trading network leading to the now burgeoning coastal cities. Cowpea suffers from a number of different biotic constraints, but insect pests inflict the most substantial losses (Murdock et al. 2008). Low-resource farmers face a pest control paradox: cowpea growers typically (1) do not have a supply of quality insecticides, (2) nor pesticide sprayers, (3) nor the money to buy required quantities, (4) nor the know-how to use them safely and efficiently (Murdock et al. 2008). When farmers do have resources, it is common to spray cowpea up to four times a season for effective control (Murdock et al. 2008). Across Africa the use of pesticides is increasing rapidly. Unfortunately, pesticides are frequently mislabeled, fraudulent or use chemistries that are either deregistered or banned in more progressive countries (Jepson et al. 2014; Donald et al. 2016). All these factors make it difficult for farmers to safely and effectively control the pest with yields as low as one-tenth of the yield potential (Murdock et al. 2008). The impoverished cowpea farmers of Africa, a great many of whom are women, stand empty handed and largely helpless against the ravenous insects that take their crop. Traditional breeding/screening research to bring needed insect resistance into cowpea cultivars suitable for the region has had little success against the major insect pests of cowpea, especially for the legume pod borer (LPB), *Maruca vitrata* Fabricius (Lepidoptera, Crambidae) (Murdock et al. 2008). However, Bt δ-endotoxins (Cry proteins) of the soil bacterium *Bacillus thuringiensis* (Bt) subsp. *kurstaki* are highly toxic against *M. vitrata* early-instar larvae (Srinivasan 2008). A major advance in the management of LPB has been achieved by engineering cowpea to encode genes that express a Cry1Ab delta endotoxin of *B. thuringiensis* (Popelka et al. 2006). Multiyear Confined Field Trials (CFT) in Burkina Faso, Ghana and Nigeria show that genetically engineered (GE) Bt-cowpea plants encoding a Cry1Ab protein are immune to LPB (Addae et al. 2007, Submitted). Once released to farmers, Bt-cowpea should protect cowpea from yield loss resulting from LPB feeding.

Despite its promise and the acute need for Bt-cowpea, there are many hurdles to its adoption. Before GE Bt-cowpea can be released to African farmers, it must receive regulatory approval from the African regulatory authorities in countries where it will be grown (Huesing et al. 2011). In addition, a seed system is currently being developed for sustainable production of high-quality Bt-cowpea seed including a quality management system that can ensure the technology meets international Stewardship guidelines (http://www.excellencethroughstewardship.org/). For regulatory approval, Bt-cowpea will undergo a rigorous environmental risk assessment (ERA) process. In part, the ERA will estimate the probability of a harmful effect to non-target organisms or to the environment that might result from the deployment of Bt-cowpea in farmers’ fields in sub-Saharan Africa.

The ERA of all GE plants, including Bt-cowpea, does not focus on generating new scientific knowledge but instead serves as an aid to decision making (Hill and Sendashonga 2003; Romeis et al. 2009). The ERA process is designed to answer very specific, relevant and realistic questions about the potential risks of introducing Bt-cowpea plants into the African environment (Romeis et al. 2008, 2011; Raybould 2010). Requirements for new assessment data are limited to those necessary to reach a confident conclusion of acceptable risk. If suitable data on which to base a regulatory decision are already available, they can and should be used to inform the risk assessment. Requests for additional data merely to answer interesting scientific questions should be avoided (Raybould 2007b). In other words, risk assessments should be based on “need to know” not “nice to know” data.

A potential environmental risk from a GE insect-resistant crop is that desirable organisms, primarily arthropods (often referred to by the general term “non-target organism” or NTO), will be harmed by exposure to the insecticidal protein. A useful approach to framing the ERA is the formation of an “Expert Panel” to assess available data and identify data gaps prior to initiation of the formal risk assessment. In that light, a Bt-cowpea expert panel was convened in 2009 which addressed six specific questions associated with the potential environmental risk of Bt-cowpea to NTOs (Huesing et al. 2011). Briefly, the panel determined that for NTOs exposed to Cry1Ab and Cry2Ab[1] in cowpea the current safety data and history of safe use of Cry1A and Cry2 class proteins provide important NTO safety data for Bt-cowpea expressing Cry1Ab. The panel also determined that the currently known expression profile of Cry1Ab in cowpea effectively removes some NTO groups from consideration since these organisms would have limited or no exposure to Cry1Ab. The panel also outlined the data likely needed to support the familiarity component of the registration package including field assessments of select NTOs collected in the product development and regulatory registration phases. A key outcome of the expert panel and the subject of the present paper is the assessment of potential effects on non-target organisms given the likelihood of gene flow from conventional to wild cowpea. The expert panel

[1] This review also considers the use of Cry2Ab, where appropriate, because a second-generation Bt-cowpea is under development which will use a *cry2Ab* gene for insect resistance management.

recommended further assessment of published papers and institutional reports as well as field survey work to determine whether any unique species might be exposed and harmed by Cry1Ab or Cry2Ab proteins in wild cowpea.

Potential uses of Bt-cowpea

Widespread deployment of Bt-cowpea in the heart of the cowpea-growing area of sub-Saharan Africa has the potential to significantly increase grain yields without increasing insecticide use on the crop; indeed, it will result in less insecticide use while protecting against yield losses. The magnitude of the protection will vary with the location and the degree of LPB pressure in any particular year. Estimates of losses to LPB vary but are always substantial. In Taiwan, for example, Liao and Lin (2000) estimated losses to LPB to be 17–53% based on increased pod yields after insecticide treatment. Likewise, in West Africa LPB caused yield losses of 25–80%, although it is difficult to isolate specific losses due to *M. vitrata* from losses caused by other insects like thrips and pod-sucking bugs (Singh et al. 1990; Echendu and Akingbohungbe 1990). Increasing cowpea yields will bring benefits to both producers and consumers in the cowpea-growing and cowpea-consuming nations making up the Nigerian grain shed (Langyintuo and Lowenberg-DeBoer 2006). Long-term deployment of Bt-cowpea will increase yields while lowering the market price of the grain (Langyintuo and Lowenberg-DeBoer 2006). For growers, the lowered market price will be compensated by higher yields. Relative shares of the benefits for producers and consumers will vary with location and whether the country is a net cowpea importer or exporter (Langyintuo and Lowenberg-DeBoer 2006). The overall benefit will include a greater and more stable supply of cowpea grain for consumers, increased incomes for farmers and reduced exposure of growers, consumers and the environment to insecticides.

The Bt-Cowpea arthropod food web

To evaluate which natural enemies might be exposed to insecticidal proteins and may be at risk in GE cowpea fields in Africa, an arthropod food web was compiled to identify and prioritize non-target organisms (NTO; primarily arthropods) for risk assessment using the approach similar to that described by Romeis et al. (2009, 2014a, b), and Li et al. (2017). The basis for the food web was peer-reviewed literature retrieved from the Purdue University Library (West Lafayette, Indiana, USA) and the ISI Web of Science. Rare books and unpublished reports were obtained from the library of the International Institute of Tropical Agriculture, IITA, at Ibadan in Nigeria. IITA is the sole CGIAR center with a global mandate for research on cowpea. Our primary focuses were those arthropods routinely reported in the literature.

The target pest of Bt-cowpea: *Maruca vitrata* F

Distribution

The legume pod borer, *M. vitrata* (Syn *Maruca testulalis*), is a pest of grain legumes in the tropics and subtropics (Taylor 1967; Raheja 1974). The wider distribution of this insect extends from the Cape Verde Islands in West Africa to Fiji and the Samoa Islands in the Far East including Australia and Southeast Asia. The pest has also been reported in the West Indies and Americas. It has never been recorded in Europe or in the Mediterranean basin. The geographic origin of *M. vitrata* is controversial. Molecular analyses suggest different subspecies of *M. vitrata* worldwide (Margam et al. 2011a, b, c) with additional subpopulations in West Africa (Margam et al. 2011b; Agunbiade et al. 2012), although the most recent study confirms a possible Southeast Asian origin (Periasamy et al. 2015).

Biology and ecology

The complete life cycle of LPB requires from 22 to 25 days (Singh and Jackai 1988) depending on the temperature. Adults emerge throughout the day, but the greatest emergence occurs at night (Huang and Peng 2001; Lu et al. 2007). Emergence of both sexes is almost synchronous, and the sex ratio is 1:1. Adult *M. vitrata* are nocturnal (Lu et al. 2007), and the mating frequency peaks in 3-day-old females (Huang and Peng 2001; Lu et al. 2007). Females prefer high humidity (> 80%) and moderate temperatures (20 to 24 °C) for mating. Females typically only mate once (Atachi and Gnanvossou 1989), while males may have multiple matings (Jackai et al. 1990). The gravid female oviposits preferably on flower buds, but the eggs may be deposited singly or in batches of 2–6 on vegetative buds, on flowers and sometimes on leaf axils (Bruner 1930; Wolcott 1933; Krishnamurthy 1936; Taylor 1967, 1978). A single female may lay 200 to 800 eggs within 3 to 14 days depending on environmental conditions (Taylor 1967, 1978; Akinfenwa 1975; Huang and Peng 2001; Chi et al. 2005; Naveen et al. 2009). Hatching occurs 3 to 5 days after eggs are laid and young larvae feed on the tender parts of stems, peduncles, flowers, flower buds and young pods (Singh and Jackai 1988; Atachi and Gnanvossou 1989), but infestation is more prevalent in flowers (Chi et al. 2003). Larvae are nocturnal (Usua and Singh 1979), typically feed inside of the cleistogamous cowpea flower where they are protected from environmental stressors, and move from one flower to another as they grow. Each larva may consume 4–6 flowers before larval development is completed (Gblagada 1982). Larvae of *M. vitrata* are dispersed randomly on the flowers of a cowpea plant (Firempong and Mangalit 1990). Third- to fifth-instar larvae are capable of boring into the pods, where they are protected from environmental stressors, and occasionally into the peduncle and stems (Taylor 1967). There are five larval stages, and larval development is completed within 8 to 14 days depending on the environmental conditions (Singh and Allen 1980; Okeyo-Owuor and Ochieng 1981; Singh and Jackai 1985). There is a pre-pupal stage of 1–2 days (Taylor 1978). Pupation occurs in a silken cocoon attached to the plant or inside the pods and lasts 5–14 days (Ochieng et al. 1981; Singh and Jackai 1985). *M. vitrata* does not undergo diapause, and populations of the insect during the off season are maintained on a wide range of host plants (Okeyo-Owuor and Ochieng 1981) including 23 species of Fabaceae in Benin (Arodokoun et al. 2003) and 13 species in western Burkina Faso (Traore et al. 2014). Three to four generations of *M. vitrata* can occur on cowpea annually with additional generations surviving on alternative host plants particularly in the humid southern part of its range (Adati et al. 2012).

The availability of alternative hosts and thus the survival of *M. vitrata* populations vary along a south–north gradient. In the humid and moist tropical south host plants are abundant year-round and *M. vitrata* is endemic. Endemic *M. vitrata* emigrates from this humid zone to cowpea-cropping areas in the north following the intertropical convergence zone. In the arid northern Sahelian Savanna, where the dry season typically lasts 7 to 8 months, the number of alternative hosts diminishes rapidly and *M. vitrata* occurs only as a seasonal migratory pest during the rainy season and generally becomes locally extinct (Bottenberg et al. 1997; Ba et al. 2009; Margam et al. 2011a, b; Onstad et al. 2012). There is a middle zone between the humid south and the arid north where a patchwork of *M. vitrata* populations exist on alternative hosts plants, especially along rivers. This is consistent with data reported in northern Nigeria (Bottenberg et al. 1997) and in Burkina Faso (Ba et al. 2009; Margam et al. 2011a, b, c; Traore et al. 2014).

Crop damage

M. vitrata is a key cowpea pest because the larvae feed on the tender parts of the stem, peduncles, flower buds, flowers and pods (Singh and Jackai 1988). The extent of losses to *M. vitrata* is difficult to assess since several other insects also feed on cowpea. However, damage to a single flower can lead to the loss of one potential pod. Larval density of one *M. vitrata* larva per flower is enough to cause significant yield losses, and a single larva may consume around five flowers during development to adulthood (Atachi and Ahohuendo 1989). Yield losses range from 25 to 80% depending on the agro-ecological region, prevailing climatic conditions and cowpea variety (Echendu and Akingbohungbe 1990; Singh et al. 1990).

Non-target arthropod pests of cowpea

Cowpea is attacked by numerous insect pests during all stages of its growth and development. Insect pests include species from the following orders: Lepidoptera, Hemiptera, Coleoptera and Thysanoptera (Singh et al. 1990). The Cry1Ab and Cry2Ab proteins expressed in Bt-cowpea specifically target lepidopteran pests (CERA 2011, 2013; OECD 2007). Accordingly, they are not expected to affect other non-lepidopteran pests such as thrips, pod-sucking bugs and aphids.

Lepidoptera species

In addition to *M. vitrata* several other lepidopteran species have been observed on cowpea. These include the cowpea seed moth *Cydia ptychora* Meyrick (Lepidoptera: Tortricidae), the hairy caterpillar *Amsacta moorei* Butler (Lepidoptera: Noct.), the cotton bollworm *Helicoverpa armigera* Hübner (Lepidoptera: Noct.) *and Spodoptera* spp. (Lepidoptera: Noctuidea) (Jackai and Daoust 1986; Singh and Van Emden 1979) including the newly introduced invasive species, *Spodoptera frugiperda*, the Fall Armyworm (Meagher et al. 2004). There have been no reports of feeding by any known charismatic lepidopteran pests (http://biologie.ens-lyon.fr/ressources/bibliographies/m1-11-12-biosci-reviews-ducarme-f-1c-m.xml). Like *M. vitrata*, *C. ptychora* also feed on cowpea floral parts and pods, but in addition it also feeds on leaf buds (Olaifa and Akingbohungbe 1981). In Senegal, *A. moorei* occasionally causes serious damage on cowpea (Ndoye 1978; Bal 1991), while across Africa, *H. armigera* and *Spodoptera* spp. are minor or sporadic pests on cowpea (Jackai and Adalla 1997). *A. moorei* feeds only on cowpea leaves, but *H. armigera* and *Spodoptera* spp. feed on leaves and pods (Jackai and Daoust 1986). In northern Nigeria, lycaenids are found feeding on cowpea year-round but are most common in the dry season unlike *M. vitrata,* which is primarily a wet season pest. Lycaenid infestations rarely have a measurable effect on cowpea yield (Bottenberg et al. 1997).

Thysanoptera species

Thrips are among the most widespread insect pests of cowpea in West Africa. Several thrips species have been reported on cowpea, including the legume foliage thrips, *Hydatothrips adolfifriderici* Karny, *Frankliniella schultzei* Moulton and *Megalurothrips sjostedti* Trybom (Okwakpam and Youdeowei 1980; Ezueh 1981; Jackai and Daoust 1986;

Bottenberg et al. 1998). Among these three species, *M. sjostedti* is the only species that seriously threatens cowpea production in Africa (Salifu 1992; Tamò et al. 1993a). Damage is caused by the nymphs, which feed on the flower buds and the flowers leading to necrosis and abscission (Tamò et al. 1993b). Severely infested plants do not produce any flowers, and yield losses ranging between 20 and 100% have been reported (Singh and Allen 1980).

Hemiptera species

Aphididae and Cicadellidae Aphids and Cicadellidae are the only homopteran species encountered on cowpea in West Africa. The aphid *Aphis craccivora* K. is a widespread insect pest of cowpea in West Africa (Singh and Van Emdem 1979; Ofuya 1987; Bottenberg et al. 1998) occurring after a period of drought. It damages the plant by sucking phloem sap and transmitting viral diseases (Jackai and Daoust 1986). The insect feeds on young leaves, stems, terminal shoots and petioles of seedlings and, as the plants mature, moves to pods and flowers (Jackai and Daoust 1986; Ofuya 1989). Heavy feeding causes the stunting of the plants leading to leaf distortion, premature defoliation and death of seedlings (Singh and Allen 1980). Heavy infestation can seriously affect yield (Ofuya 1989).

Empoasca sp. are the most common species of leafhoppers encountered on cowpea (Parh and Taylor 1981). However, it is a minor pest of cowpea in West Africa (Singh and Van Emden 1979; Jackai and Daoust 1986; Bottenberg et al. 1998).

Heteroptera species In West Africa, cowpea pods suffer damage by a complex of Heteropteran species, commonly known as pod-sucking bugs (PSBs). Among these are, *Aspavia* spp. *Nezara viridula* L., *Riptortus dentipes* Fab., *Anoplocnemis curvipes* Fab., *Mirperus jaculus* Thun. and *Clavigralla tomentosicollis* Stål. Among the complex of PSBs, *C. tomentosicollis* constitutes 80% of the heteropteran populations in Nigeria and Burkina Faso (Suh et al. 1986; Dabire 2001). Adults and nymphs suck the sap from developing pods and seeds and cause premature drying and abscission of pods, seed malformation or total seed abortion. Grain yield losses may reach 60–100% in the absence of an effective control measures (Singh and Jackai 1985).

Coleoptera species

Several coleopterans feed on cowpea including the leaf beetle, *Ootheca sp* and blister beetles, *Mylabris sp*. The leaf beetle damage is done by the adults which feed between veins of the leaves. Dense populations can totally defoliate cowpea seedlings, resulting in the death of the plant. The blister beetles feed on cowpea flowers. Beetles are minor pests of cowpea in Africa, and the damage caused by direct feeding is insignificant unless their populations are extremely high (Singh and Jackai 1985).

The cowpea seed beetle, *Callosobruchus maculatus*, is the principal post-harvest pest of cowpea. Damage occurs to seeds during storage. The unprotected seeds can be completely destroyed after six months of storage (Ouedraogo et al. 1996). Infestations start in the field when the pods are maturing (Huignard et al. 1985; Sanon et al. 2005).

The beneficial arthropod fauna: predators, parasitoids and bees

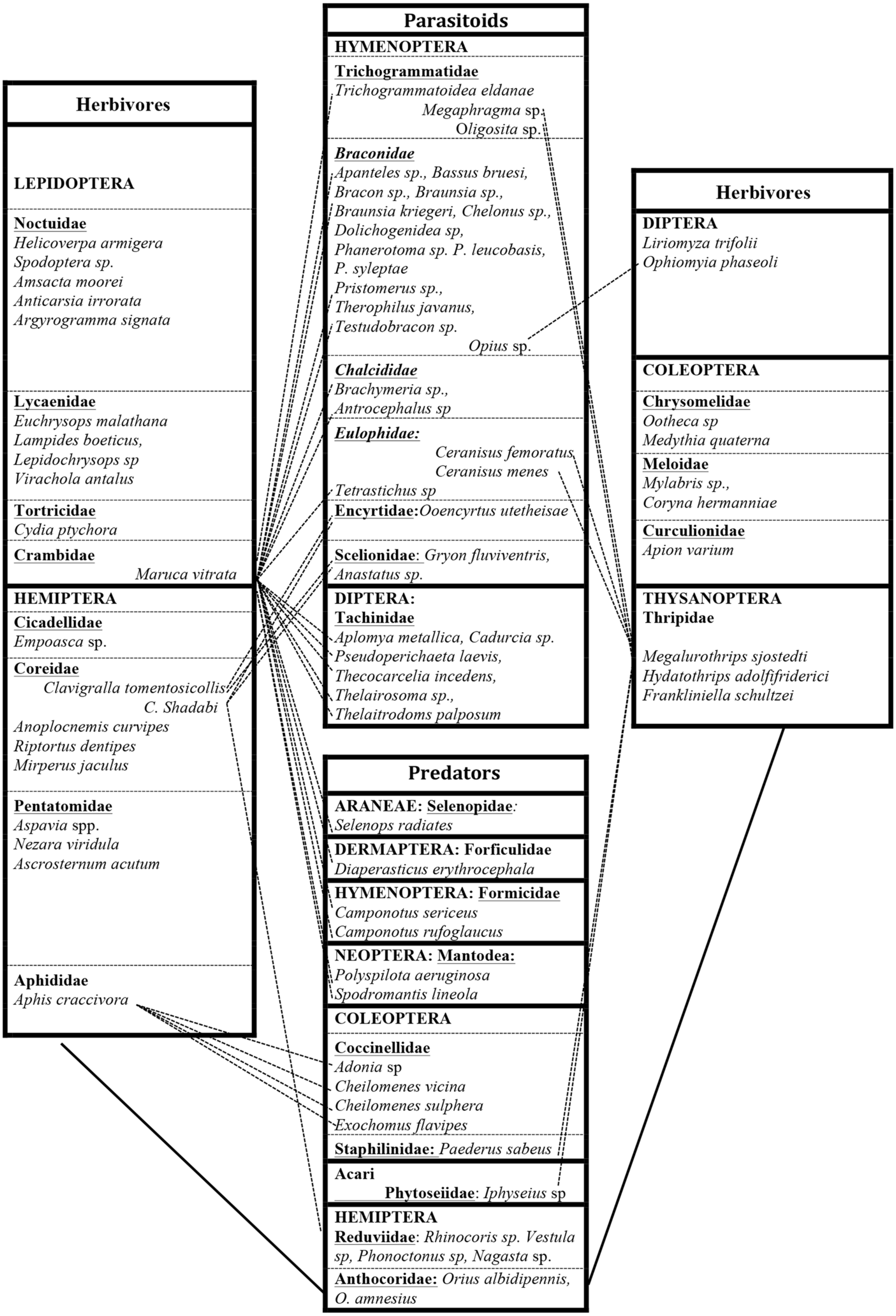
Natural enemies of cowpea insect pests (predators and parasitoids)

As in all cropping systems there are a variety of parasitoids and predators that attack cowpea pests (Fig. 1). Hymenopteran parasitoids that attack thrips eggs and/or larvae include species from the Eulophidae and Trichogrammatidae families (Tamò et al. 1993b; Adati et al. 2007; Tamò et al. 2012). Thrips predators include mites from the Phytoseiidae family as well as beetles from the Coccinellidae and Staphylinidae families. Hemipteran predators include mainly *Orius* sp. (Tamò et al. 1993b; Bottenberg et al. 1998; Adati et al. 2007).

Several dipteran parasitoid species from the family Tachinidae attack *M. vitrata* larvae (Agyen-Sampong 1978; Usua and Singh 1979; Okeyo-Owuor et al. 1991; Arodokoun et al. 2006; Adati et al. 2007). In addition, larval parasitoids from the hymenopteran family Braconidae are prevalent (Taylor 1967; Agyen-Sampong 1978; Usua and Singh 1979; Okeyo-Owuor et al. 1991; Arodokoun et al. 2006; Adati et al. 2007). Two braconids from Southeast Asia are currently being evaluated for their biological control potential against *M. vitrata* in West Africa (Tamò et al. 2016). Hymenoptera pupal (Chalcididae), larval (Eulophidae) and egg (Trichogrammatidae) parasitoids have also been observed (Usua and Singh 1979; Okeyo-Owuor et al. 1991; Arodokoun et al. 2006; Adati et al. 2007). Predators that feed on larval and adult *M. vitrata* include spiders (Selenopidae), ants (Formicidae) and mantids (Mantidae) (Usua and Singh 1979; Adati et al. 2007). The hymenopteran parasitoid *Gryon fulviventris* (Scelionidae) is the only beneficial recorded that attacks the pod bug *Clavigralla tomentosicollis* (Asante et al. 2000; Adati et al. 2007).

Cowpea floral bees

In West Africa, several bees forage on cowpea flowers including honey bees *Apis mellifera adonsonii* (Fatokoun and Ng 2007; Fohouo et al. 2009; Ige et al. 2011). In addition, carpenter bees, *Xylocopa* sp, digger bees, *Anthophora*


Herbivores
LEPIDOPTERA
Noctuidae
Helicoverpa armigera
Spodoptera sp.
Amsacta moorei
Anticarsia irrorata
Argyrogramma signata
Lycaenidae
Euchrysops malathana
Lampides boeticus,
Lepidochrysops sp
Virachola antalus
Tortricidae
Cydia ptychora
Crambidae
Maruca vitrata
HEMIPTERA
Cicadellidae
Empoasca sp.
Coreidae
Clavigralla tomentosicollis
C. Shadabi
Anoplocnemis curvipes
Riptortus dentipes
Mirperus jaculus
Pentatomidae
Aspavia spp.
Nezara viridula
Ascrosternum acutum
Aphididae
Aphis craccivora
Parasitoids
HYMENOPTERA
Trichogrammatidae
Trichogrammatoidea eldanae
Megaphragma sp.
Oligosita sp.
Braconidae
Apanteles sp., Bassus bruesi,
Bracon sp., Braunsia sp.,
Braunsia kriegeri, Chelonus sp.,
Dolichogenidea sp,
Phanerotoma sp. P. leucobasis,
P. syleptae
Pristomerus sp.,
Therophilus javanus,
Testudobracon sp.
Opius sp.
Chalcididae
Brachymeria sp.,
Antrocephalus sp
Eulophidae:
Ceranisus femoratus
Ceranisus menes
Tetrastichus sp
Encyrtidae: Ooencyrtus utetheisae
Scelionidae: Gryon fluviventris,
Anastatus sp.
DIPTERA:
Tachinidae
Aplomya metallica, Cadurcia sp.
Pseudoperichaeta laevis,
Thecocarcelia incedens,
Thelairosoma sp.,
Thelaitrodoms palposum
Predators
ARANEAE: Selenopidae:
Selenops radiates
DERMAPTERA: Forficulidae
Diaperasticus erythrocephala
HYMENOPTERA: Formicidae
Camponotus sericeus
Camponotus rufoglaucus
NEOPTERA: Mantodea:
Polyspilota aeruginosa
Spodromantis lineola
COLEOPTERA
Coccinellidae
Adonia sp
Cheilomenes vicina
Cheilomenes sulphera
Exochomus flavipes
Staphilinidae: Paederus sabeus
Acari
Phytoseiidae: Iphyseius sp
HEMIPTERA
Reduviidae: Rhinocoris sp. Vestula sp, Phonoctonus sp, Nagasta sp.
Anthocoridae: Orius albidipennis, O. amnesius
Herbivores
DIPTERA
Liriomyza trifolii
Ophiomyia phaseoli
COLEOPTERA
Chrysomelidae
Ootheca sp
Medythia quaterna
Meloidae
Mylabris sp.,
Coryna hermanniae
Curculionidae
Apion varium
THYSANOPTERA
Thripidae
Megalurothrips sjostedti
Hydatothrips adolfifriderici
Frankliniella schultzei

◂**Fig. 1** Web food table of herbivores that feed upon cowpea in West Africa (Adati et al. 2007; Bottenberg et al. 1998; Jackai and Daoust 1986; Singh and Allen 1980; Singh and Van Emden 1979; Dreyer 1994)

sp., bumble bees, *Bombus sp.* leaf-cutting bees, *Megachile* sp. have been reported in West Africa (Pasquet et al. 2007; Asiwe 2009a, b; Fohouo et al. 2009).

Insect pests of wild cowpea

Wild *Vigna* species have been extensively monitored in West Africa for occurrence of cultivated cowpea insect pests (Taylor 1967; Atachi and Djihou 1994; Arodokoun et al. 2003). Most of the wild cowpea species, *Vigna racemosa, Vigna vexillata,* and *V. unguiculata* subspecies *spontanea* (formerly *dekindtiana*) host *M. vitrata* and flower thrips, *Megalurothrips sjostedti* (Jackai et al. 1996; Bottenberg et al. 1998; Arodokoun et al. 2003). Among the *Vigna* species, some are known to be highly resistant to *M. vitrata* (Jackai et al. 1996) thanks to both antixenosis and antibiosis mechanisms. Importantly, since the wild *V. unguiculata* subspecies *spontanea* (formerly *dekindtiana*) is the only subspecies that can interbreed with cultivated cowpea (Fatokoun 2002; Kouadio et al. 2007), the wild species *V. racemosa* and *V. vexillata* need not be considered further in the risk assessment.

Assessing potentially harmful non-target effects of Bt-cowpea

Before commercial deployment of an insecticidal GE crop can begin, an environmental risk assessment (ERA) is conducted to determine the level of risk to non-target organisms (NTO) (Huesing et al. 2011). NTOs include non-target beneficial arthropods and threatened and endangered animals (Mendelsohn et al. 2003; OECD 2007; USEPA 2007). In addition, non-target pests may be assessed especially as they relate to issues of IRM or potential crop weediness. The ERA for Bt-cowpea will be conducted following internationally recognized approaches (Romeis et al. 2011). Data requirements for risk assessment arise during the problem formulation stage, the first stage of the risk assessment process (Romeis et al. 2008). This stage determines the purpose and scope of the risk assessment and guides subsequent data collection (Romeis et al. 2009). Most importantly, in the problem formulation stage those entities or processes needing protection (the protection goals), e.g., protection of pollinators or pest predators, are identified. Once the protection goals are identified, the risk assessment of Bt-cowpea concentrates on estimating the likelihood of harm resulting from the introduced *cry1ab* and *cry2Ab* genes (Raybould 2007a). In addition to outlining protection goals, problem formulation also develops the assessment endpoints and testable risk hypotheses used to test for potential harm to the protected entity. Together, these data lead to characterization of the risk to the protected entity (Raybould 2007b). While the NTO ERA is conducted for the environment in which the GE crop will be grown, the uniformity and harmonization of the process allows for substantial data transportability, i.e., data from international tests conducted on the transgene and protein of interest, e.g., Cry1Ab and Cry2Ab, can be used in regulatory submissions throughout the world, and in this case, sub-Saharan Africa (Romeis et al. 2009; Raybould and Quemada 2010). Specific studies conducted in an African country can likewise be used in other (African and international) countries.

Protection of biodiversity, specifically NTOs, is one of the management goals defined by countries that are signatories to the Convention on Biological Diversity (SCBD 2000). Because insecticidal GE crops target insect pests, an important part of the ERA is their potential impact on NTOs including organisms providing important ecological services such as biological control of herbivores and pollination (Romeis et al. 2008, 2009; Huesing et al. 2011).

Implications for non-target risk assessment of insecticidal GE cowpea

Worldwide, governmental regulatory agencies have evaluated Cry1A and Cry2A containing GE crops to determine the potential for direct or indirect toxic effects on non-target birds, mammals, humans and arthropods including beneficial insects found in and around agricultural fields including threatened endangered species found in the USA (Mendelsohn et al. 2003) or Europe (EFSA 2009; CERA 2011, 2013).

The safety assessment of Cry1A- and Cry2A-containing crops is based on: (i) an understanding of several criteria including the mode of action and specificity of Bt Cry toxins; (ii) direct testing of NTOs in feeding bioassays; and (iii) the long history of safe use of Bt Cry toxins both as insecticidal sprays (Federici et al. 2007) as well as *in planta* expression (OECD 2007).

The weight of evidence shows that Cry1A and Cry2A toxins have a very narrow activity spectrum targeting insects in the order Lepidoptera (OECD 2007; CERA 2010, 2011, 2013). Safety assessments of the Cry1A (both Cry1Ab and Cry1Ac) and Cry2A toxins have shown that their insecticidal activity is highly specific for lepidopterans (Huesing et al. 2011; CERA 2010, 2011, 2013). The toxicity and specificity of the Cry1A and Cry2A proteins are associated with their solubilization and proteolytic activation in the digestive tracts of susceptible lepidopteran insects. Following solubilization in the lepidopteran's slightly basic (~pH 8.0) digestive fluid the toxins bind to specific epithelial brush border membrane receptors. These receptors only occur in the midguts of susceptible lepidopteran insects (OECD 2007; Pigott and Ellar 2007). Since these Cry receptors are not present in non-target birds, mammals and humans these proteins pose essentially no hazard to non-lepidopteran organisms (Wolfersberger et al. 1986; Hofmann et al. 1988; Van Rie et al. 1989, 1990; Shimada et al. 2006a, b; OECD 2007).

Before they were commercially released into the environment, Cry1A and Cry2A class toxins were extensively tested in the laboratory by the technology developer. GE crops expressing the Cry proteins or the proteins themselves were tested against a broad range of pest and beneficial arthropods including species from the Orders Coleoptera, Diptera, Hemiptera, Homoptera, Hymenoptera, Lepidoptera, Orthoptera, Neuroptera, Isoptera, the insect relatives collembola and the crustacean order Isopoda to assess potential toxic effects (OECD 2007; Romeis et al. 2008; Raybould 2007a; CERA 2010, 2011, 2013). These tests were conducted under international regulatory guidelines at toxin concentrations generally exceeding 10X the expected environmental concentration (EEC) (Mendelsohn 2003; OECD 2007; CERA 2010; USEPA 2007, 2010, 2011). Extensive testing was also independently conducted in academic laboratories to assess the safety of Cry1A and Cry2A Bt toxins against NTO beneficial insects including the predators *Chrysoperla carnea* (Neuroptera: Chrysopidae) and *Hippodamia convergens* (Coleoptera: Coccinellidae), the parasitic wasp *Nasonia vitripennis* (Hymenoptera: Pteromalidae), and soil organisms including the collembolan *Folsomia candida* (Collembola: Isotomidae) and the earthworms *Aporrectodea caliginosa* and *Lumbricus terrestris* (Haplotaxida: Lumbricidae) (Romeis et al. 2006; OECD 2007; Wolfenbarger et al. 2008; Naranjo 2009) as well as the honey bee *Apis mellifera* (Hymenoptera: Apidae) (Duan et al. 2008). Data sets collected from field studies have also been analyzed using meta-analysis at the taxonomic and functional guild levels to support the conclusion of safety of these proteins (OECD 2007; Wolfenbarger et al. 2008; Marvier et al. 2007). It appears that the two Cry proteins have a high degree of specificity and have no effect on organisms outside the order Lepidoptera (OECD 2007; Romeis et al. 2008). As part of the risk assessment process, the Cry1Ab and Cry2Ab proteins expressed in cowpea will be assessed for functional equivalency against the same proteins expressed in other Bt crops and if expression levels are comparable, the non-target risk assessment data collected for other Bt crops can be used in the risk assessment for Bt-cowpea. Since cowpea and other commercialized Bt crops harbor similar groups of beneficial arthropods, it is reasonable to conclude that cowpea plants expressing Cry1Ab and Cry2Ab will pose little risk to NTOs and there is thus no need for additional non-target risk assessment studies.

Since gene flow is expected to occur between cultivated Bt-cowpea and wild cowpea plants (Huesing et al. 2011), NTOs will likely be exposed to the Cry1Ab and Cry2Ab proteins outside of cultivated cowpea. Part of the review process conducted here aims to establish whether unique taxa exist and are exposed to the Bt toxins outside of cultivation to determine whether additional testing may be required to address the risk to these organisms. Since cowpea is a self-pollinated (cleistogamous) crop, exposure to other NTOs will be limited to NTOs that feed on the plant and possibly to bees that might ingest Cry1Ab and Cry2Ab contained in pollen. Fortunately, the safety of Cry1Ab and Cry2Ab to the order Hymenoptera including honey bees (Duan et al. 2008), and for Cry1Ab only, bumble bees (Babendreier et al. 2008) and solitary bees (Konrad et al. 2008) is well established. In addition, only the largest bees, e.g., carpenter bees, are able to penetrate the cowpea flower further reducing the number of organisms that are exposed to Cry1Ab and Cry2Ab (Coulibaly et al. 2002; Pasquet et al. 2007, 2008).

The Simplified Food Web

Risk to non-target organisms is a function of (i) exposure and (ii) toxicity (Romeis et al. 2008). Accordingly, when formulating risk hypotheses NTOs not exposed to Cry proteins expressed in Bt-cowpea can be excluded from the analysis allowing the risk assessment to focus on those NTOs that are exposed. The simplified arthropod food web for cowpea fields presented above (Fig. 1) can be used to identify which NTOs would be exposed either directly or indirectly to insecticidal Cry1Ab and Cry2Ab proteins in Bt-cowpea (Romeis et al. 2009). Importantly, in this simplified food web not every insect occurring in cowpea is necessarily listed (though the assessment was exhaustively compiled from both the formal and informal literature), but care was taken to ensure that every functional group is well represented and adequate for a robust risk assessment (Romeis et al. 2009). In addition, based on evidence to date as well as the near taxonomic relatedness of wild and cultivated cowpea it is highly unlikely that NTO species occurring in cultivated cowpea would not be present in wild cowpea and vice versa.

While several lepidopteran species feed on cowpea, with the exception of LPB, rarely cause economic damage. These lepidopteran species include a tortricid species as well as a variety of noctuids including *Helicoverpa* and *Spodoptera* spp. Importantly, unsubstantiated field reports have observed the newly introduced spodopteran pest, fall armyworm, on cowpea, but it is not expected to be a serious pest as larvae develop poorly on the crop (Meagher et al. 2004). As mentioned, lycaenids are routinely found feeding on cowpea, but they too rarely reach pest status (Bottenberg et al. 1997). In no instance has a charismatic lepidopteran species been observed on cowpea in Africa. Accordingly, Bt-cowpea is expected to have negligible impact on lepidopteran species other than the intended target of the technology the LPB.

Based on the risk assessment of all previous Bt-expressing GE crops it is unlikely that phloem-feeding arthropods such as aphids and planthoppers would be exposed to the Bt proteins in cowpea, regardless of their inherent toxicity, since there is no evidence that Bt proteins are transported in the phloem sap of Bt-expressing crops (Lawo et al. 2009; Romeis et al. 2009; Huesing et al. 2011). Accordingly, aphid predators such as coccinellid adults and larvae are unlikely to be at risk because exposure is negligible. Likewise, there would be no Cry exposure to egg parasitoids, since insect eggs have not been found to contain any Cry proteins (Romeis et al. 2013). Thus, only predators or parasitoids that directly consume plant material or that attack herbivores that have fed on plant tissue are likely to be exposed to the insecticidal Cry1Ab and Cry2Ab proteins. Included in this group are predatory and herbivorous beetles such as ladybird beetles and hemipterans such as *Oruius* spp. Studies of effects of Cry1Ab on the ladybird beetles *Propylea japonica* and *Hippodamia convergens* established the No Effect Level (NOEL). NOELs of > 500 ppm were observed for *Propylea* and > 20 ppm for *Hippodamia* (Table 1). Likewise, a NOEL > 20 ppm was established for the parasitic hymenopteran *Brachymeria intermedia* (Table 1). Thus, at expression levels observed in Bt-cowpea we can expect negligible effects on these functional groups.

Hymenoptera serve a variety of important ecological functions including their role as parasitoids (e.g., Ichneumonidae), predators (e.g., Eumenidae) and pollinators (Apidae) and could therefore be exposed to insecticidal Cry proteins via several routes. The cleistogamous nature of cowpea was thought to largely limit all but the largest carpenter bees from exposure to Bt-cowpea pollen (Pasquet et al. 2007). New evidence shows that honey bees also pollinate cowpea (Aiswe 2009a, b). Larval parasitoids would be exposed to Cry proteins as a result of parasitizing herbivores feeding on Bt-cowpea. At the dietary exposure levels of Cry proteins in Bt-cowpea event 709A (up to 5.3 ng/mg of plant tissue) (Table 2) effects on larval parasitoids would be highly unlikely. Indeed, the potential toxic effects of Cry1Ab and Cry2Ab on bees has been exhaustively studied with the conclusion of no negative effects at Cry levels far exceeding those observed in Bt-cowpea pollen (Tables 1 and 2) (OECD 2007).

Likewise, predatory omnivorous hemipteran bugs, e.g., *Orius* spp., feed on both pest insects and plant tissues (pollen and young leaves) and are thus potentially exposed to higher toxin doses as compared to other, obligate, predators, but all studies to date show no adverse effects on these insects at doses far exceeding what they would encounter in Bt-cowpea (Corey et al. 1998; Duan et al. 2007; Lundgren et al. 2008). Likewise, all studies to date show no adverse effects of Cry proteins against Neuroptera species (Table 1).

Summary

In summary, we show here that using information collected from the African agricultural areas where Bt-cowpea will be released ("the receiving environment") together with information on the toxicity and mode of action of Cry1Ab and Cry2Ab proteins, we can formulate reasonable testable risk hypotheses to assess the potential effects of Cry1Ab and Cry2Ab expressed in Bt-cowpea and deployed in West Africa. More importantly we can use this information to rule out the need for a number of costly studies that would add little information to the safety assessment of a Bt-cowpea.

The long history of safe use of Bt toxins generally, and the Cry1Ab and Cry2Ab toxins specifically, both as sprayable insecticides and as genes expressed in GE crops, suggests strongly that these proteins are specific for lepidopteran larvae and have not been shown to cause harm to other arthropod species, birds, fish or mammals (CERA 2010, 2011, 2013; OECD 2007). Any assessment can thus focus only on lepidopteran arthropod species that are exposed, either through feeding on cowpea plant tissues or on prey that have fed on cowpea tissues. All lepidopteran insects feeding on cowpea are classified as pests, and none are listed or considered as threatened or endangered.

The beneficial fauna observed in cowpea are predominately comprised of common non-target beneficial arthropods found throughout the world's agricultural systems. These include hymenopteran parasitoids that attack thrips eggs and larvae as well as predators of thrips such as mites and common Coccinellidae and Staphylinidae beetles. Hemipteran *Orius* sp. predators are encountered as well. The coleopteran predators and *Orius* sp. are known to feed on a variety of food sources including larval lepidopterans. A variety of parasitoids known to attack lepidopteran insects such as LPB larvae is also present in cowpea fields and includes dipteran tachinids and hymenopteran braconids, Chalcididae, Eulophidae and Trichogrammatidae. Spiders, ants and mantids have also been routinely observed. Based on the broad literature review conducted here we conclude that there are no unique arthropod species in the likely deployment area for Bt-cowpea in Africa that have not been assessed elsewhere for other GE crops. Since these non-target risks for Cry1Ab and Cry2Ab have already been assessed in a different context (different plants, different regions) no additional non-target testing

Table 1 Effect of Cry1Ab and Cry2A proteins on selected non-target insect species

Insect order	Non-target species	Type of cry protein	Dosage	Response variable	Toxicity	Reference
Coleoptera	*Propylea japonica*	Cry2Ab provided in artificial diet	500 µg/ml	Nymphal development	No adverse effect	Zhao et al. (2016)
Coleoptera	*Hippodamia convergens*	Cry1Ab single dose	NOEL > 20 ppm	Mortality	No adverse effect	CERA (2011)
Hemiptera	*Orius majusculus*	Cry1Ab supplied by leaves, pollen or phytophagous insect	Unspecified	Survival, development, fecundity and fertility	No adverse effect	Lumbierres et al. (2012)
Hemiptera	*Orius tantilus*	Cry1Ab rice-fed thrips along with Cry1Ab rice pollen	Unspecified	Nymphal duration, adult longevity, and female fecundity	No adverse effect	Raen et al. (2016)
Hemiptera	*Orius insidiosus*	The predator is given *Thrips tabaci* fed with Cry2Ab cotton leaves	43.637 ng/mg	Nymphal development	No adverse effect	Kumar et al. (2014)
Homoptera	*Aphis gossypii*	Cry2Ab provided in artificial diet	500 µg/ml	Nymphal development	No adverse effect	Zhao et al. (2016)
Hymenoptera	*Apis mellifera*	Stacked Cry1Ac/Cry2Ab provided in cotton pollen	(up to 92 ng/g)	Nymphal development	No adverse effect	Niu et al. (2013)
Hymenoptera	*Apis mellifera*	Cry1Ab Unspecified	Unspecified	Adult and larval mortality	No adverse effect	Duan et al. (2008)
Hymenoptera	*Apis mellifera*	Cry1Ab/Unspecified	20 ppm	Adult and larval mortality	No adverse effect	Duan et al. (2008)
Hymenoptera	*Apis mellifera*	Cry2A/Unspecified	50 µg/ml	Adult and larval mortality	No adverse effect	Duan et al. (2008)
Hymenoptera	*Brachymeria intermedia*	Cry1Ab single dose in diet	NOEL > 20 ppm	Adult mortality	No adverse effect	CERA (2011)
Diptera	*Exorista civilis*	The parasitoid is given *Mythimna separata* larvae fed with up to 25 µg/g of Cry1Ab	Unspecified	Parasitism and parasitoid development	No adverse effect	Jiang et al. (2016)
Lepidoptera	*Bombyx mori*	Stacked Cry1Ac/Cry2Ab provided in cotton pollen	9.2 ng/ml)	Nymphal development	No adverse effect	Niu et al. (2013)
Neuroptera	*Chrysoperla carnea*	Pollen of Bt maize expressing Cry1Ab	5 ng/ml	Adult survival and female fecundity	No adverse effect	Li et al. (2008)
Neuroptera	*Chrysoperla carnea*	Pollen of Bt maize expressing Cry1Ab	Unspecified	Adult survival and female fecundity	No adverse effect	Romeis et al. (2014a, b)
Neuroptera	*Chrysoperla rufilabris*	Supplied with larvae of *Trichoplusia ni* and *Spodoptera frugiperda* fed on stacked Cry1Ac/Cry2Ab produced in Bt cotton	Unspecified	Adult and larval survival and female fecundity	No adverse effect	Tian et al. (2013)

data should be required to come to a conclusion of negligible risk. However, consultation with regional regulators will determine whether the available non-target data on Cry1Ab and Cry2Ab together with field surveys of the arthropod fauna in Bt-cowpea fields in the region will be sufficient for a regulatory assessment.

By making effective use of all relevant data that have been generated previously for the regulatory approval of other GE crops expressing these same insecticidal proteins regulatory approvals for Bt-cowpea in West Africa can be expedited without compromising safety and without incurring extra unneeded costs (Huesing et al. 2011; Kalaitzandonakes et al.

Table 2 Cry1Ab protein expression in different organs from T5 generation transgenic cowpea line 709A

	Cry1Ab ng/per mg fresh weight tissue	
	Conventional variety IT86D-716	Transgenic Event 709A derived from IT86D-716
Roots	0	0.25 ± 0.07
Leaf	0	5.3 ± 2.2
Flower	0	4.5 ± 0.6
Pod	0	3.9 ± 0.4
Green cotyledon	0	2.1 ± 0.6
Dry seed	0	2.5 ± 0.2
Pollen	0	0.12 ± 0.08
Anther wall	0	1.2 ± 0.2

(Expression of the Cry1Ab protein in transgenic cowpea line 709A has been quantified using Agdia Bt Cry1Ab/1Ac ELISA kits. By including a protein standard curve using purified bacterial Cry1Ab protein supplied by Monsanto, an accurate quantification of Cry1Ab expression can be calculated) (Personal communication from Higgins TJ)

2007). The focus of any regulatory testing can address any remaining potentially significant risks or uncertainties. For Bt-cowpea this would likely be confirmatory field assessments made on wild cowpeas capable of crossing with the Bt-cowpea variety as well as collection of the standard "familiarity" data that are assessed on all GE crops in the field testing stage of product development. Familiarity data establish the level of similarity of ecologically and agronomically relevant characteristics between the GE crop and its non-transformed comparator. Generally, the familiarity data are collected as part of the regulatory agronomic assessment that 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 GE crops will also be collected for Bt-cowpea and will lead to a conclusion of minimal risk for the introduction of Bt-cowpea into West Africa.

Author contributions

All authors participated in the drafting of this paper as individual subject matter experts in their fields, and the authors are solely responsible for the content. Any views expressed in this paper are the views of the authors and do not necessarily represent the views of any organization, institution or government with which they are affiliated or employed. The authors declare no competing financial interest.

Acknowledgements This work was supported by the US Department of Agriculture Foreign Agriculture Service (USDA/FAS) Norman E. Borlaug International Agricultural Science and Technology Fellowship Program (Borlaug Fellowship Program) under Sponsor Award/Grant Number: 58-3148-2-188/106117 to Purdue University. The award was to LLM through Purdue University and administered through International Programs in Agriculture. The authors are also grateful to the African Agriculture Technology Foundation (AATF) for supporting the Bt-cowpea project, which generated the data on Cry1Ab protein expression in cowpea.

References

Adati T, Tamò M, Yusuf SR, Downham MCA, Singh BB, Hammond W (2007) Integrated pest management for cowpea–cereal cropping systems in the West African savannah. Int J Trop Insect Sci 27:123–137

Adati T, Tamò M, Koji S, Downham M (2012) Effect of migration and mating status of the legume pod borer, *Maruca vitrata* (Fabricius) (Lepidoptera: Crambidae) on the efficacy of synthetic sex pheromone traps in West Africa. In: Boukar O, Coulibaly O, Fatokun C, Lopez K, Tamò M (eds) Enhancing cowpea value chains through research advances, Proceedings of the 5th World Cowpea Research Conference. IITA, Ibadan, Nigeria, pp 260–272

Agunbiade TA, Coates BS, Kim KS, Forgacs D, Margam VM, Murdock LL, Ba MN, Binso-Dabire CL, Baoua I, Ishiyaku MF, Tamò M, Pittendrigh BR (2012) The spatial genetic differentiation of the legume pod borer, *Maruca vitrata* F. (Lepidoptera: Crambidae) populations in West Africa. Bull Entomol Res 102:589–599

Agyen-Sampong M (1978) Pests of cowpea and their control in Ghana. In: Singh SR, vanEmdem HF, Taylor TA (eds) Pest of Grain Legumes: ecology and control. IITA, Ibadan, pp 85–92

Akinfenwa S (1975) Biological study of *Maruca testulalis* (Geyer) (Lepidoptera: Pyralidae) in the Zaria area of northern Nigeria. M.Sc. Thesis, Ahmadu Bello University, Nigeria

Arodokoun DY, Tamò M, Cloutier C, Adeoti R (2003) The importance of alternative host plants for the annual cycle of the legume pod borer, *Maruca vitrata* Fabricius (Lepidoptera: Pyralidae). Insect Sci Appl 23:103–113

Arodokoun DY, Tamò M, Cloutier C, Brodeur J (2006) Larval parasitoids occurring on *Maruca vitrata* Fabricius (Lepidoptera: Pyralidae) in Benin, West Africa. Agr Ecosyst Environ 113:320–325

Asante SK, Jackai LEN, Tamò M (2000) Efficiency of *Gryon fulviventris* (Hymenoptera: Scelionidae) as an Egg Parasitoid of *Clavigralla tomentosicollis* (Hemiptera: Coreidae) in Northern Nigeria. Environ Entomol 29: 815–821

Asiwe JAN (2009). Insect mediated outcrossing and geneflow in cowpea (*Vigna unguiculata* (L.) Walp): implication for seed production and provision of containment structures for genetically transformed cowpea. Afr J Biotechnol 8(2):226–230, ISSN 1684–5315 © 2009 Academic Journals

Asiwe JAN (2009b) Insect mediated outcrossing and geneflow in cowpea (Vigna *unguiculata* (L.) Walp): Implication for seed production and provision of containment structures for genetically transformed cowpea. Afr J Biotechnol 8:226–230

Atachi P, Ahohuendo BC (1989) Comparaison de quelques paramètres caractéristiques de la dynamique des populations entre *Megalurothrips sjostedti* (Trybom) et *Maruca testulalis* (Geyer) sur une même plante hôte, le niébé. Insect Sci Appl 10:187–197

Atachi P, Djihou ZC (1994) Record of the host plants of *Maruca testulalis* (Geyer) (Lepidoptera: Pyralidae) in the Republic of Benin. Ann Soc Entomol Fr 30:169–174

Atachi P, Gnanvossou D (1989) Dynamique quantitative des populations animales: recherches préliminaires à une étude comparée des dynamiques de biomasses, d'effectifs et de production chez

Maruca testulalis (Geyer) (Lep. Pyralidae) en culture de niébé dans un agrosystème du Sud Benin. Acta Oecol 10:221–239

Addae PC, Ishyiaku M, Tignegre JB, Bationo J, Atokple IDK, Saba M, Onyekachi FN, Lawan M, Huesing J, Beach L, Higgins TJV (2017) Efficacy of cry1Ab gene to control the pod borer *Maruca vitrata* in cowpea. Submitted

Ba NM, Margam VM, Dabire-Binso CL, Sanon A, McNeil J, Murdock LL, Pittendrigh BR (2009) Seasonal and regional distribution of the cowpea pod borer, *Maruca vitrata* Fabricius (Lepidoptera: Crambidae), in Burkina Faso. Int J Trop Insect Sci 29:109–113

Babendreier D, Reichhard B, Romeis J, Bigler F (2008) Impact of insecticidal proteins expressed in transgenic plants on bumblebee micro-colonies. Entomol Exp Appl 126:148–157

Bal AB (1991) Action threshold for flower thrips on cowpea *(Vigna unguiculata* (L.) Walp.) in Senegal. Trop Pest Manage 37:363–367

Bottenberg H, Tamò M, Arodokoun D, Jackai LEN, Singh BB, Youm O (1997) Population dynamics and migration of cowpea pests in northern Nigeria: implications for integrated pest management. In: Singh BB, Mohan-Raj DR, Dashiell KE, LE Jackai N (eds) Advances in Cowpea Research. International Institute of Tropical Agriculture and Japan International Center for Agricultural Sciences, Ibadan, pp 271–284

Bottenberg H, Tamò M, Singh BB (1998) Occurrence of phytophagous insects on wild *Vigna* sp. and cultivated cowpea: comparing the relative importance of host-plant resistance and millet intercropping. Agr Ecosyst Environ 70:217–229

Bruner SC (1930) Report of the department of entomology and phytopathology for 1929–1930. Experiment Station of Santiago de Las Vegas, Cuba, p 74

CERA (2010) A review of the environmental safety of the Cry1Ac protein. Center for Environmental Risk Assessment. ILSI Research Foundation 1–18

CERA (2011) A review of the environmental safety of the Cry1Ab Protein. Center for Environmental Risk Assessment. ILSI Research Foundation 1–17

CERA (2013) A Review of the Environmental Safety of the Cry2Ab Protein. Center for Environmental Risk Assessment. ILSI Research Foundation 1–25

Chi Y, Sakamaki Y, Tsuda K, Kusigemachi K (2003) The seasonal abundance of the Legume Pod Borer, *Maruca vitrata* in Kagoshima, Japan. Mem Fac Agr Kagoshima Univ 38:41–44

Chi Y, Sakamaki Y, Tsuda K, Kusigemati K (2005) Effect of temperature on oviposition and adult longevity of the legume pod borer, *Maruca vitrata* (Fabricius) (Lepidoptera: Crambidae). Appl Entomol Zool 49:29–32

Corey D, Kambhampati S, Wilde G (1998) Electrophoretic analysis of *Orius insidiosus* (Hemiptera: Anthocoridae) feeding habits in field corn. J Kansas Entomol Soc 71:11–17

Coulibaly S, Pasquet RS, Papa R, Gepts P (2002) AFLP analysis of the phenetic organization and genetic diversity of *Vigna unguiculata* L. Walp. reveals extensive gene flow between wild and domesticated types. Theor Appl Genet 104:358–366

Dabire LCB (2001) Etude de quelques parametres biologiques et ecologiques de *Clavigralla tomentosicollis* Stal 1855 (Hemiptera: Coreidae), punaise suceuse des gousses de niebe [*Vigna unguiculata* (L.) Walp.] dans une perspective de lutte durable contre l'insecte au Burkina Faso. PhD Thesis dissertation, Universite de Cocody, Abidjan, Cote d'Ivoire

Donald CE, Scott RP, Blaustein KL, Halbleib ML, Sarr M, Jepson PC, Anderson KA (2016) Silicone wristbands detect individuals' pesticide exposures in West Africa. R Soc Open Sci 3:160433. https://doi.org/10.1098/rsos.160433

Dreyer H (1994) Seed damaging field pests of cowpea (*Vigna unguiculata*) in southern Benin, with special reference to *Clavigralla tomentosicolis* Stal (Het., Coreidae). PhD Thesis No. 10894, Swiss Federal Institute of Technology Zurich

Duan JJ, Huesing J, Teixeira D (2007) Development of tier-I toxicity assays for *Orius insidiosus* (Heteroptera: Anthocoridae) for assessing the risk of plant-incorporated protectants to nontarget heteropterans. Environ Entomol 36:982–988

Duan JJ, Marvier M, Huesing JE, Dively G, Huang ZY (2008) A meta-analysis of effects of Bt crops on honey bees (Hymenoptera: Apidae). PLoS ONE. https://doi.org/10.1371/journal.pone.0001415

Echendu TNC, Akingbohungbe AE (1990) Intensive free–choice and no choice cohort tests for evaluating resistance to Maruca testulalis (Lepidoptera:Pyralidae) in cowpea. Bull Entomol Res 80:289–293

EFSA (2009) Scientific Opinion of the Panel on Genetically Modified Organisms on applications (EFSA-GMORXMON810) for the renewal of authorisation for the continued marketing of (1) existing food and food ingredients produced from genetically modified insect resistant maize MON810; (2) feed consisting of and/or containing maize MON810, including the use of seed for cultivation; and of (3) food and feed additives and feed materials produced from maize MON810, all under Regulation (EC) No 1829/2003 from Monsanto. EFSA J 1149:1–85

Ezueh MI (1981) Nature and significance of preflowering damage by thrips to cowpea. Entomol Exp Appl 29:305–312

FAOSTAT (2017). http://www.fao.org/faostat/en/#data/QC/visualize (retrieved on 07 September 2017)

Fatokun CA (2002) Breeding cowpea for resistance to insect pests: attempted crosses between cowpea and *Vigna vexillata*. In: Fatokun CA, Tarawali SA, Singh BB, Kormawa PM, Tamò M (eds) Challenges and opportunities for enhancing sustainable cowpea production, Proceedings of the 3rd World Cowpea Conference. International Institute of Tropical Agriculture, Ibadan, Nigeria, pp 52–61

Fatokun CA, Ng Q (2007) Outcrossing in cowpea. J Food Agr Environ 5:334–338

Federici B (2007) Bacteria as biological control agents for insects: economics, engineering and environmental safety. In: Vurro M, Gressel J (eds) Novel Biotechnologies for Biocontrol agent enhancement and management. Springer, Berlin, pp 25–51

Firempong S, Mangalit H (1990) Spatial distribution of *Maruca testululis* larvae on cowpea, and a sequential sampling plan for estimating larval densities. Insect Sci Appl 11:217–222

Fohouo FNT, Ngakou A, Kengni BS (2009) Pollination and yield responses of cowpea (Vigna *unguiculata* L. Walp.) to the foraging activity of *Apis mellifera adansonii* (Hymenoptera: Apidae) at Ngaoundéré (Cameroon). Afr J Biotechnol 8:1988–1996

Gblagada CCS (1982) Inventaire des parasites lavaires de *Maruca testulalis* (Geyer) sur le niebe (Vigna *unguiculata* (L.) Walp) et sur le pois d'angole (Cajanus *cajan* L. Millsp.). MS Dissertation Universite Abomey-Calavi, Benin

Hill RA, Sendashonga C (2003) General principles for risk assessment of living modified organisms: lessons from chemical risk assessment. Environ Biosafety Res 2:81–88

Hofmann C, Vanderbruggen H, Höfte H, Van Rie J, Jansens S, Van Mellaert H (1988) Specificity of *Bacillus thuringiensis* delta-endotoxins is correlated with the presence of high-affinity binding sites in the brush border membrane of target insect midguts. Proc Natl Acad Sci 85:7844–7848

Huang CC, Peng WK (2001) Emergence, mating and oviposition of the bean pod borer, *Maruca vitrata* (F.) (Lepidoptera: Pyralidae). Formosan Entomol 21:37–45

Huesing J, Romeis J, Ellstrand N et al (2011) Regulatory considerations surrounding the deployment of Bt-expressing cowpea in Africa: report of the deliberations of an expert panel. GM Crops 2:211–224

Huignard J, Leroi B, Alzouma I, Germain JF (1985) Oviposition and development of *Bruchidius atrolineatus* and *Callosobruchus maculatus* (Coleoptera: Bruchidae) in *Vigna unguiculata* cultures. Insect Sci Appl 6:691–699

Ige OE, Olotuah OF, Akerele V (2011) Floral biology and pollination ecology of cowpea (*Vigna unguiculata* L. Walp). Modern Appl Sci 5:74–82

Jackai LEN, Adalla CB (1997) Pest management practices in cowpea: a review. In: Singh BB, Mohan-Raj DR, Dashiell KE, Jackai LEN (eds) Advances in cowpea research. International Institute of Tropical Agriculture and Japan International Center for Agricultural Sciences, Ibadan, pp 240–258

Jackai LEN, Daoust RA (1986) Insect pests of cowpeas Ann Rev Entomol 31:95–119

Jackai LEN, Ochieng RS, Raulston JR (1990) Mating and oviposition behavior of the legume pod borer, *Maruca testulalis*. Entomol Exp Appl 59:179–186

Jackai LEN, Padulosi S, Ng Q (1996) Resistance to the legume pod borer, *Maruca vitrata* Fabricius, and the probable modalities involved in wild *Vigna*. Crop Prot 15:753–761

Jepson PC, Guzy M, Blaustein K, Sow M, Sarr M, Mineau P, Kegley S (2014) Measuring pesticide ecological and health risks in West African agriculture to establish an enabling environment for sustainable intensification. Phil Trans R Soc B 369:2013049. https://doi.org/10.1098/rstb.2013.0491

Jiang XF, Zhang L, Yang H, Sappington TH, Cheng Y, Luo LZ (2016) Biocontrol of the oriental armyworm, *Mythimna separata* by the tachinid fly *Exorista civilis* is synergized by Cry1Ab protoxin. Sci Rep 6:26873. https://doi.org/10.1038/srep26873

Kalaitzandonakes N, Alston JM, Bradford KJ (2007) Compliance costs for regulatory approval of new biotech crops. Nat Biotechnol 25:509–511

Konrad R, Ferry N, Gatehouse AMR, Babendreier D (2008) Potential effects of oilseed rape expressing Oryzacystatin-1 (OC-1) and of purified insecticidal proteins on larvae of the solitary bee *Osmia bicornis*. PLoS ONE. https://doi.org/10.1371/journal.pone.0002664

Kouadio D, Echikh N, Toussaint A, Pasquet RS, Baudoin JP (2007) Organisation du pool génique de *Vigna unguiculata*(L.) Walp.: croisements entre les formes sauvages et cultivées du niébé. Biotechnol Agron Soc Environ 11:47–57

Krishnamurthy B (1936) The Avare pod borers, a new method of control. Mysore Agricultural Calendar and Year Book, Bangalore

Kumar R, Tian JC, Naranjo SE, Shelton AM (2014) Effects of Bt cotton on *Thrips tabaci* (Thysanoptera: Thripidae) and its predator, *Orius insidio*sus (Hemiptera: Anthocoridae). J Econ Entomol 107:927–932

Langyintuo A, Lowenberg-DeBoer J (2006) Potential regional trade implications of adopting Bt cowpea in West and Central Africa. Ag Bio Forum 9:111–120

Langyintuo AS, Lowenberg-DeBoer J, Faye M, Lambert D, Ibro G, Moussa B, Kergna A, Kushwaha S, Musa S, Ntoukam G (2003) Cowpea supply and demand in West and Central Africa. Field Crop Res 82:215–231

Lawo NC, Wäckers FL, Romeis J (2009) Indian *Bt* Cotton varieties do not affect the performance of cotton Aphids. PLoS One 4:e4804. https://doi.org/10.1371/journal.pone.0004804

Li Y, Meissle M, Romeis J (2008) Consumption of Bt maize pollen expressing Cry1Ab or Cry3Bb1 does not harm adult green lacewings, *Chrysoperla carn*ea (Neuroptera: Chrysopidae). PLoS One 3(8):e2909. https://doi.org/10.1371/journal.pone.0002909

Li Y, Zhang Q, Liu Q, Meissle M, Yang Y, Wang Y, Hua H, Chen X, Peng Y, Romeis J (2017) Bt rice in China—focusing the nontarget risk assessment. Plant Biotechnol J 15:1340–1345

Liao CT, Lin CS (2000) Occurrence of the legume pod borer, *Maruca testullis*, Geyer (Lepidoptera: Pyralidae) on cowpea (*Vigna unguiculata* Walp) and its insecticidal application trial. Plant Prot Bull 42:213–222

Lu PF, Qiao HL, Wang XP, Wang XQ, Lei CL (2007) The emergence and mating rhythms of the legume pod borer, *Maruca vitrata* (Fabricius, 1787) (Lepidoptera: Pyralidae). Pan-Pac Entomol 83:226–234

Lumbierres B, Albajes R, Pons X (2012) Positive effect of Cry1Ab-expressing Bt maize on the development and reproduction of the predator *Orius majusculus* under laboratory conditions. Biol Control 63:150–156

Lundgren JG, Fergen JK, Riedell WE (2008) The influence of plant anatomy on oviposition and reproductive success of the omnivorous bug *Orius insidiosus*. Anim Behav 75:1495–1502

Margam VM, Coates BS, Ba MN, Sun W, Binso-Dabire CL et al (2011a) Geographic distribution of phylogenetically-distinct legume pod borer, *Maruca vitrata* (Lepidoptera: Pyraloidea: Crambidae). Mol Biol Rep 38:893–903

Margam VM, Coates BS, Hellmich RL, Agunbiade T et al (2011b) Mitochondrial genome sequence and expression profiling for the legume pod borer *Maruca vitrata* (Lepidoptera: Crambidae). PLoS ONE. https://doi.org/10.1371/journal.pone.0016444

Margam VM, Coates BS, Bayles DO et al. (2011c) Transcriptome sequencing, and rapid development and application of SNP markers for the legume pod borer *Maruca vitrata* (Lepidoptera: Crambidae) PLoS One. https://doi.org/10.1371/journal.pone.0021388

Marvier M, McCreedy C, Regetz J, Kareiva P (2007) A meta-analysis of effects of Bt cotton and maize on non-target invertebrates. Science 316:1475–1477

Meagher RL, Nagoshi RN, Stuhl C, Mitchell ER (2004). Larval development of Fall Armyworm (Lepidoptera: Noctuidae) on different crop plants. Florida Entomol 87(4):454–460. Doi: 10.1653/0015-4040(2004)087[0454:LDOFAL]2.0.CO;2

Mendelsohn M, Kough J, Vaituzis Z, Matthews K (2003) Are Bt crops safe? Nat Biotechnol 21:1003–1009

Murdock LL, Coulibaly O, Higgins TJV, Huesing JE, Ishiyaku MF, Sithole-Niang I (2008) Cowpea: Legume grains and forages. In: Kole C, Hall TC (eds) A compendium of transgenic crop plants. Blackwell Publishing, Oxford, pp 23–56

Naranjo SE (2009) Impacts of Bt crops on non-target invertebrates and insecticide use patterns. CAB Rev Perspect Agric Vet Sci Nutr Nat Resour 4:23. https://doi.org/10.1079/PAVSNNR20094011

Naveen V, Naik MI, Manjunatha M, Shivanna BK, Sridhar S (2009) Biology of legume pod borer, *Maruca testulalis* Geyer on cowpea. Karnataka J Agr Sci 22:668–669

Ndoye M (1978) Pests of cowpea and their control in Senegal. In: Singh SR, Van Emden HF, Taylor TA (eds) Pests of grain legumes: ecology and control. International Institute of Tropical Agriculture, Ibadan, pp 113–115

Niu L, Ma Y, Mannakkara A, Zhao Y, Ma W, Lei C, Chen L (2013) Impact of single and stacked insect-resistant bt-cotton on the honey bee and silkworm. PLoS One 8(9):e72988. https://doi.org/10.1371/journal.pone.0072988

Ochieng RS, Okeyo-Owuor JB, Dabrowski ZT (1981) Studies on the legume pod borer, Maruca-Testulalis (Geyer): mass rearing on natural food. Insect Sci Applic 1:269–272

OECD (2007) Consensus document on safety information on transgenic plants expressing *Bacillus thuringiensis*. OECD Environment, Health and Safety Publications Series on Harmonization of Regulatory Oversight in Biotechnology. ENV/JM/MONO (2007)14: OECD 2007

Ofuya TI (1987) A population explosion of *Aphis craccivora* Koch (Homoptera: Aphididae) in cowpeas protected with cypermethrin. FAO Plant Prot Bull 35:75–77

Ofuya TI (1989) The effect of pod growth stages in cowpea on aphid reproduction and damage by the cowpea aphid, *Aphis craccivora* (Homoptera: Aphididae). Ann Appl Biol 115:563–566

Okeyo-Owuor JB, Ochieng R (1981) Studies on the legume pod-borer *Maruca testululis* (Geyer)–1: Life cycle and behavior. Insect Sci Appl 1:263–268

Okeyo-Owuor JB, Oloo GW, Agwaro PO (1991) Natural enemies of the legume pod borer, *Maruca testulalis* Geyer (Lepidoptera: Pyralidae) in small scale farming systems of western Kenya. Insect Sci Appl 12:35–42

Okwakpam BA, Youdeowei A (1980) The annotated key to four species of thrips (Thysanoptera) attacking edible legumes in Nigeria. Bull IFAN 42:157–165

Olaifa JI, Akingbohungbe AE (1981) Aspects of the biology of the black cowpea moth, *Cydia ptychora* (Lepidoptera: Tortricidae) related to host plant phenology. Ann Appl Biol 97:129–134

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

Ouedraogo AP, Sou S, Sanon A, Monge JP, Huignard J, Tran MD, Credland PF (1996) Influence of temperature and humidity on populations of *Callosobruchus maculatus* (Coleoptera: Bruchidae) and its parasitoid *Dinarmus basalis* (Pteromalidae) in two zones of Burkina Faso. Bull Entomol Res 86:695–702

Parh IA, Taylor TA (1981) Studies on the life cycle of the cicadellid bug *Empoasca dolichi* Paoli, in Southern Nigeria. J Nat Hist 15:829–835

Pasquet R, Pittendrigh B, Ishiyaku M, Baoua I, Dabire C, Ba M, Huesing J, Murdock L (2007) Addressing gene flow issues in cowpea for West Africa. North Cent Weed Sci Soc Proc 62:81

Pasquet RS, Peltier A, Hufford MB et al (2008) Long-distance pollen flow assessment through evaluation of pollinator foraging range suggests transgene escape distances. Proc Natl Acad Sci USA 105:13456–13461

Periasamy M, Schafleitner R, Muthukalingan K, Ramasamy S (2015) Phylogeographical structure in mitochondrial DNA of legume pod borer (*Maruca vitrata*) population in tropical Asia and sub-Saharan Africa. PLoS ONE. https://doi.org/10.1371/journal.pone.0124057

Pigott CR, Ellar DJ (2007) Role of receptors in *Bacillus thuringiensis* crystal toxin activity. Microbiol Mol Biol Rev 71:255–281

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

Raen AZ, Dang C, Wang F, Peng YF, Ye GY (2016) Thrips-mediated impacts from transgenic rice expressing Cry1Ab on ecological fitness of non-target predator *Orius tantilus* (Hemiptera: Anthocoridae). J Integr Agri 15:2059–2069

Raheja AJ (1974) Report on the insect pests of grain legumes in northern Nigeria. In: Proceedings, 1st IITA Grain Legume Improvement Workhop. International Institute of Tropical Agriculture, Ibadan, Nigeria, pp 295–299

Raybould A (2007a) Environmental risk assessment of genetically modified crops: general principles and risks to non-target organisms. BioAssay 2:8

Raybould A (2007b) Ecological versus ecotoxicological methods for assessing the environmental risks of transgenic crops. Plant Sci 173:589–602

Raybould A (2010) Reducing uncertainty in regulatory decision-making for transgenic crops. More ecological research or clearer environmental risk assessment? GM Crops 1:25–31

Raybould A, Quemada H (2010) Bt crops and food security in developing countries: realised benefits, sustainable use and lowering barriers to adoption. Food Secur 2:247–259

Romeis J, Meissle M, Bigler F (2006) Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. Nat Biotechnol 24:63–71

Romeis J, Bartsch D, Bigler F et al (2008) Assessment of risk of insect-resistant transgenic crops to nontarget arthropods. Nat Biotechnol 26:203–208

Romeis J, Lawo NC, Raybould A (2009) Making effective use of existing data for case-by-case risk assessments of genetically engineered crops. J Appl Entomol 133:571–583

Romeis J, Hellmich R, Candolfi M et al (2011) Recommendations for the design of laboratory studies on non-target arthropods for risk assessment of genetically engineered plants. Transgenic Res 20:1–22

Romeis J, Raybould A, Bigler F, Candolfi M, Hellmich R, Huesing J, Shelton A (2013) Deriving criteria to select arthropod species for laboratory tests to assess the ecological risks from cultivating transgenic crops. Chemosphere 90:901–909

Romeis J, Meissle M, Alvarez-Alfageme F, Bigler F, Bohan DA, Devos Y, Malone LA, Pons X, Rauschen S (2014a) Potential use of an arthropod database to support the non-target risk assessment and monitoring of transgenic plants. Trangenic Res 23:995–1013

Romeis J, Meissle M, Naranjo SE, Li Y, Bigler F (2014b) The end of a myth-Bt (Cry1Ab) maize does not harm green lacewings. Front Plant Sci 5:391. https://doi.org/10.3389/fpls.2014.00391

Salifu AB (1992) Some aspects of the biology of the bean flower thrips *Megalurothrips sjostedti* (Trybom) (Thysanoptera: Thripidae) with reference to economic injury levels on cowpea (*Vigna unguiculata* (L.) Walp). Rev Zool Afr 106:451–459

Sanon A, Dabiré C, Ouedraogo AP, Huignard J (2005) Field occurrence of bruchid pests of cowpea and associated parasitoids in a Sub humid zone of Burkina Faso: Importance of the infestation of two cowpea varieties at harvest. J Plant Pathol 4:14–20

SCBD (Secretariat of the Convention on Biological Diversity) (2000) Cartagena protocol on biosafety to the convention on biological diversity: text and annexes. SCBD, Montreal

Shimada N, Miyamoto K, Kanda K, Murata H (2006a) *Bacillus thuringiensis* insecticidal Cry1Ab toxin does not affect the membrane integrity of the mammalian intestinal epithelial cells: An in vitro study. In Vitro Cell Dev-An 42:45–49

Shimada N, Murata H, Mikami O et al (2006b) Effects of feeding calves genetically modified corn Bt11: a clinicobiochemical study. J Vet Med Sci 68:1113–1115

Singh SR, Allen DR (1980) Pests, diseases, resistance, and protection in cowpea. In: Summerfield RJ, Bunting AH (eds) Advances in Legume Science. HMSO, London, pp 419–443

Singh SR, Jackai LEN (1985) Insect pests of cowpeas in Africa: their life cycle, economic importance and potential for control. In: Singh SR, Rachie KO (eds) Cowpea research, production and utilization. Wiley, Chichester, pp 217–231

Singh SR, Jackai LEN (1988) Mini Review. The legume pod-borer, *Maruca testulalis* (Geyer): Past, present and future research. Insect Sci Appl 9:1–5

Singh SR, Van Emden HF (1979) Insect pests of grain legumes. Annu Rev Entomol 24:255–278

Singh SR, Jackai LEN, Dos Santos JHR, Adalla CB (1990) Insect pests of cowpea. In: Singh SR (ed) Insect pests of tropical food legumes. Wiley, Chichester, pp 43–89

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

Suh JB, Jackai LEN, Hammond WNO (1986) Observations on pod sucking bug populations on cowpea at Mokwa, Nigeria. Trop Grain Legume Bull 33:17–19

Tamò M, Baumgaertner J, Delucchi V, Herren HR (1993a) Assessment of key factors responsible for the pest status of the bean flower thrips *Megalurothrips sjostedti* (Thysanoptera, Thripidae) in West Africa. Bull Entomol Res 83:251–258

Tamò M, Baumgaertner J, Arodokoun DY (1993b) The spatio temporal distribution of *Megalurothrips sjostedti* (Thysanoptera, Thripidae) life stages on cowpea, and development of sampling plans. Bull Soc Entomol Suisse 66:15–34

Tamò M, Srinivasan R, Dannon E, Agboton C, Datinon B, Dabire C, Baoua I, Ba MN, Haruna B, Pittendrigh BR (2012) Biological control: a major component for the long-term cowpea pest Management strategy. In: Fatokun CA, Tarawali SA, Singh BB, Kormawa PM, Tamò M (eds) Challenges and opportunities for enhancing sustainable cowpea production, Proceedings of the 3rd World Cowpea Conference. International Institute of Tropical Agriculture, Ibadan, Nigeria, pp 249–259

Tamò M, Pittendrigh BR, Miresmailli S, Font V, Blalock B, Dannon E, Datinon B, Agyekum M, Donovan C, Biaou E (2016) From biocontrol to precision-IPM in Africa: Challenges and opportunities. International Congress of Entomology, Orlando, Florida, 24–28 Sept, 2016. Abstract 0644: https://doi.org/10.1603/ice.2016.95128

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

Taylor TA (1978) *Maruca testulalis*, an important pest of tropical grain. In: Singh SR, Van Emden HF, Taylor TA (eds) Pests of grain legumes: ecology and control. Academic Press, London, pp 193–200

Tian J-C, Wang X-P, Long L-P, Romeis J, Naranjo SE, Hellmich RL et al (2013) Bt crops producing Cry1Ac, Cry2Ab and Cry1F do not harm the green Lacewing. Chrysoperla rufilabris. PLoSOne 8(3):e60125. https://doi.org/10.1371/journal.pone.0060125

Traore F, Ba NM, Dabire-Binso CL, Sanon A, Pittendrigh BR (2014) Annual cycle of the legume pod borer *Maruca vitr*ata Fabricius (Lepidoptera: Crambidae) in southwestern Burkina Faso. Arthropod-Plant Inte 8:155–162

USEPA (2007) White paper on tier-based testing for the effects of proteinaceous insecticidal plant-incorporated protectants on non-target arthropods for regulatory risk assessments. Rose R. Ed. Washington, DC: US Environmental Protection Agency Washington, DC. https://www.epa.gov/sites/production/files/2015-09/documents/tier-based-testing.pdf

USEPA (2010) United States Environmental Protection Agency Biopesticide Registration Document Cry1Ab and Cry1F Bacillus thuringiensis (Bt) Corn Plant-Incorporated Protectants https://www3.epa.gov/pesticides/chem_search/reg_actions/pip/cry1f-cry1ab-brad.pdf

USEPA (2011) United States Environmental Protection Agency Biopesticide Registration Document *Bacillus thuringiensis* Cry1A.105 and Cry2Ab2 Insecticidal Proteins and the Genetic Material Necessary for Their Production in Corn [PC Codes 006515 (Cry2Ab2), 006514 (Cry1A.105)]. https://www3.epa.gov/pesticides/chem_search/reg_actions/pip/mon-89034-brad.pdf

Usua EJ, Singh SR (1979) Behavior of cowpea pod borer, *Maruca testulalis* Geyer. Nigerian J Entomol 3:231–239

Van Rie J, Jansens S, Hofte H, Degheele D, Van Mellaert H (1989) Specificity of *Bacillus thuringiensis* δ-endotoxins. Eur J Biochem 186:239–247

Van Rie J, Jansens S, Hofte H, Degheele D, Van Mellaert H (1990) Receptors on the brush border membrane of the insect midgut as determinants of the specificity of *Bacillus thuringiensis* delta-endotoxins. Appl Environ Microbiol 56:1378–1385

Wolcott GN (1933) The Lima bean pod borer caterpillars of Puerto Rico. J Dept Agr Puerto Rico 17:241–255

Wolfenbarger LL, Naranjo SE, Lundgren JG, Bitzer RJ, Watrud LS (2008) Bt crop effects on functional guilds of non-target arthropods: A meta-analysis. PLoS One 3(5):e2118. https://doi.org/10.1371/journal.pone.0002118

Wolfersberger M, Hofmann C, Luthy P (1986) Bacterial Protein Toxins. In: Falmagne P, Alouf JE, Fehrenbach FJ Jeljaszewicz J, Thelestam M (eds). Fischer, New York, 237–238

Zhao Y, Zhan S, Luo JY, Wang CY, Lv LM, Wang XP, Cui JJ, Lei CL (2016) Bt proteins Cry1Ah and Cry2Ab do not affect cotton aphid *Aphis gossypii* and ladybeetle *Propylea japonica*. Sci Rep 6:20368. https://doi.org/10.1038/srep20368