Research Application Summary

Impact of transgenic sweetpotato on non-target species

Rukarwa, R.J.[1, 3], Mukasa, S.B.[1], Odongo, B.[2], Ssemakula, G..[3], Moar, W.J.[4] & Ghislain, M.[5]
[1]School of Agricultural and Environmental Sciences, Makerere University, P. O. Box 7062,
Kampala, Uganda
[2]African Institute for Capacity Development, Kenya
[3]National Crop Resources Research Institute (NaCCRI), Namulonge, P. O. Box 7084,
Kampala, Uganda
[4] Monsanto Company, St. Louis, MO 63167
[5]International Potato Center (CIP), Nairobi, Kenya
Corresponding author: rrukarwa@agric.mak.ac.ug

Abstract

Sweetpotato is affected by the sweetpotato weevil (*Cylas* spp.) which caused immense tuber yield loss in many sub-Saharan Africa countries. Management of this pest is still difficult. The International Potato Centre has introduced insecticidal proteins derived from the bacterium *Bacillus thuringiensis* (Bt) into the crop through genetic engineering. This study has been set up to determine whether there are potential dangers of the Bt proteins to non-target beneficial insect species. Ladybird beetles (*Delphastus catalinae*), ground beetles (*Poecilus chalcites*) and rove beetles (*Aleochara bilineata*) will be collected from sweetpotato fields and used in the study. These will be fed on an artificial diet containing different Cry7Aa1 protein solutions in the laboratory. Casualties will be recorded daily for six days for the ladybird beetle and for 15 days for the remaining two non-target species. Possible impacts of transgenic sweetpotato events expressing Cry7Aa1 proteins on relevant non-target organisms (NTOs) will be determined. This information will inform environmental risk assessment decisions for the introduction of Bt sweetpotato in Africa.

Key words: *Bacillus thuringiensis*, Cry7Aa1 proteins, *Cylas* spp., environmental risk assessment, sweetpotato

Résumé

La patate douce est affectée par le charançon de la patate douce (*Cylas* spp.) qui a provoqué une immense perte de rendement en tubercules dans de nombreux pays d'Afrique sub-saharienne. La gestion de ce ravageur est encore difficile. Le Centre International de la Patate a mis en place des protéines insecticides issues de la bactérie *Bacillus thuringiensis* (Bt) dans les cultures grâce au génie génétique. Cette étude a été effectuée pour déterminer s'il existe des dangers possibles des protéines Bt pour des espèces non ciblées d'insectes bénéfiques. Les coccinelles (*Delphastuscatalinae*), les carabes (*Poeciluschalcites*) et les staphylins (*Aleocharabilineata*) seront collectés à partir des champs de patates douces et utilisés dans l'étude. Ceux-ci seront nourris avec un aliment artificiel contenant différentes solutions de protéines Cry7Aa1 dans le laboratoire. Les pertes seront enregistrées par jour pendant six jours pour la coccinelle et pendant 15 jours pour les deux autres espèces non ciblées. Les impacts possibles des événements de la patate douce transgéniques exprimant les protéines de Cry7Aa1 sur les organismes non ciblés pertinents seront déterminés. Cette information permettra d'éclairer les décisions d'évaluation des risques environnementaux pour l'introduction de la patate douce Bt en Afrique.

Mots clés: *Bacillus thuringiensis*, protéines de Cry7Aa1, *Cylas* spp., Evaluation des risques environnementaux, patate douce

Background

Sweetpotato (*Ipomoea batatas* (L.) is an important crop in East Africa, where it is grown as a staple crop (Stevenson *et al*., 2009). For some farmers, the crop also supplements family income, and thus, strategies to reduce losses to pests provide opportunities to enhance food security and improve livelihoods. For protection of the crop against the sweetpotato weevil (*Cylas* spp.), resistant plants producing insecticidal proteins derived from the bacterium *Bacillus thuringiensis* (Bt) were engineered by International Potato Center (CIP) using *Agrobacterium tumefaciens*. The sweetpotato genotypes expressing Cry7Aa1 are anticipated to provide effective protection against the sweetpotato weevil, a pest causing severe economic losses in sub Saharan Africa. Like any other pest control technologies, genetically engineered crops expressing insecticidal proteins could negatively affect beneficial species (non-target species) that provide important services to the ecosystem, and such potential negative effects should be assessed (Raybould 2007; Romeis *et al*., 2008, 2009).

Beneficial species in sweetpotato production systems include pollinators, decomposers, predators and or parasitoids of the insect pests (Table 1). The non-target species are only affected by an insecticidal compound expressed in a genetically modified crop if they ingest toxic concentrations through feeding directly on plant material, or target organisms, or through exposure to the environment; such as in the soil or water (Raybould 2007; Romeis *et al*., 2008). In general, non target species commonly found in sweetpotato fields that are related taxonomically to

Table1. Beneficial arthropods associated with Sweetpotato fields in Uganda.

Order	Family	Species	Common name	Importance	Abundance
Coleoptera	Coccinellidae	*Delphastus catalinae*	Ladybird beetle	Predator	Common
	Carabidae	*Poecilus chalcites*	Ground beetle	Predator	Common
	Staphylinidae	Aleochara bilineata	Rove beetle	Predator	Common
Hemiptera	Reduviidae	*Sycanus* spp	Assassin bug	Predator	Common
Hymenoptera	Ichneumonidae	*Charops* sp	Ichneumon wasp	Parasitoid	Common
	Braconidae	*Meteorus autographae*	Braconid wasp	Parasitoid	Common
	Apidae	*Apis mellifera*	Honey bee	Pollinator	Common
Diptera	Tachinidae	*Caricelia normula*	Tachinid fly	Parasitoid	Common
Haplotaxida	Lumbricidae	*Eisenia foetida*	Earthworm	Decomposer	Common
Araneae	Oxyopidae	*Oxyopes* spp	Lynx spider	Predator	Common
	Lycosidae	*Lycosa* sp.	Wolf spider	Predator	Common

the target pests are most likely to be affected by the toxic protein, thus selection of these taxa increases the likelihood of detecting a hazard if one exists (Romeis *et al*., 2008). In our case, beneficial species like ladybird beetles (*Delphastus catalinae*), ground beetles (*Poecilus Chalcites*) and rove beetles (*Aleochara bilineata*) have been taken into consideration as test organisms because they belong to the same order (Coleoptera) with the *Cylas* spp.

Literature Summary

Before new genetically engineered crops can be cultivated commercially, risks to human health and the environment have to be assessed and evaluated by regulatory agencies. One crucial part of the environmental risk assessment of transgenic insect-resistant crops is the evaluation of potential risks to non-target organisms (NTOs) Sanvido *et al*., 2005). NTOs are species not intended for control using a particular Cry protein expressed in transgenic plants. The focus of risk assessment is on organisms which are of importance in the ecosystem like decomposers, pollinators and biological control species. The exposure of NTOs to a Cry protein is estimated from plant expression data, the diets of non-target organisms, the rate of degradation of the protein in soil, and any other relevant environmental fate data (Raybould, 2007).

For effective NTO risk assessment, threshold values need to be defined as has been done for environmental risk assessments of conventional pesticides. If these trigger values are not exceeded, the testing stops and the regulatory decision follows. The specific triggers applied in a given case of risk assessment for insect resistant plants are informed by expert opinion and require deliberation among risk assessors and risk managers, who consider the problem being evaluated and the effects regarded as acceptable.

Study Description

The ladybird beetles, ground beetles and rove beetles are collected from sweetpotato fields at the National Crops Resources Research Institute (NaCRRI) and immediately used in bioassays upon reception. An artificial diet for predatory Coleoptera to be used in this study was previously described by Porcar *et al*. (2010). Diet consists of beef extract (60g), yeast extract (40g), sucrose (100g) and agarose (13g). These are solubilised in 800ml of sterile water by heating in a microwave oven. The mixture is cooled at room temperature before adding 65g of honey, 9g of Ain Vitamin Mixture 76 (MP Biomedical, Solon, OH, USA) and 1.1g of Nipagin. Stocks of doses, containing different Cry7Aa1 protein solutions will be tested. Three independent control treatments are prepared by adding 70 ml of water, solubilisation buffer (pH 10.5) or trypsin-treated solubilisation buffer (pH 9) to 1ml of liquid diet. All bioassays will be performed in small (5cm in diameter) Petri dishes containing a 1ml dose of artificial diet. The bioassays will be repeated three to four times in a completely randomised design at 25±1ºC, under an 18: 6 h (L: D) photoperiod. Casualties are recorded daily for six days for ladybird and for 15 days for the remaining two non-target species.

Research Application

Transgenic sweetpotato expressing Cry proteins offers the promise of safe and effective insect control unmatched by other chemical products because they are highly selective. As part of risk assessment for transgenic insect resistant crops, possible impacts of transgenic sweetpotato events expressing Cry7Aa1 proteins on relevant non-target organisms (NTOs) need to be evaluated. The present work is aimed to study the sensitivity of these predators to Cry proteins administered through artificial diet. The results will help understand the impact of Cry proteins on these beneficial Coleoptera and forecast their suitability as biological control agents in Bt sweetpotato crops.

Recommendation

The environmental risk assessment should be on a case by case basis initiated with good problem formulation. Good problem formulations minimise data requirements, while enabling risk assessments capable of indicating with high confidence that GM crops pose minimal environmental risk. Regardless of where the environmental risk assessment is conducted, the problem formulation approach needs to be very similar, using similar information that is modified by local cropping system information.

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