



Building Local Research Capacity to Support National Biosafety Systems

**Biotechnology and Biodiversity Interface (BBI)
Competitive Grant Program**

Dr. Hector Quemada (Donald Danforth Plant Science Center)

hquemada@danforthcenter.org

Dr. Karen Hokanson (University of Minnesota)

hokan018@umn.edu

The Program for Biosafety Systems

<http://www.ifpri.org/pbs/pbs.asp>

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Building Local Research Capacity to Support National Biosafety Systems

The Program for Biosafety Systems (PBS) collaborates with local and international partners to support countries in Africa and Asia in the responsible development and safe use of agricultural biotechnology. As a component of the PBS, the Biotechnology and Biodiversity Interface (BBI) Competitive Grant Program provides support for research that considers the potential effects of agricultural biotechnology on biodiversity in developing countries, particularly on biodiversity outside of agriculture, i.e. ‘wild biodiversity’. The two main goals of the BBI Program are: 1) to generate information that will be useful for regulatory decisions concerned with impacts on biodiversity, and 2) to build capacity within these countries to evaluate risks, and to design and implement risk assessment research. From 2004-2006, the BBI program selected 11 projects (**Box 1**). Each research project includes scientists from developing countries as principal investigators, and a substantial part of the research projects are conducted in one or more developing countries. Research is ongoing, and findings from these projects are being published in peer-reviewed journals.

Box 1.

| Country/Region | Focus | Objectives | Year |
|----------------------------------|--------------|--|------|
| Burkina Faso, Ethiopia and Kenya | Bt cowpea | Assess genetic diversity, genetic structure, and seed predation in wild cowpea populations. | 2005 |
| Ethiopia, Kenya and Tanzania | Rice | Investigate gene flow from crop to wild rice, fitness of hybrid progeny, and baseline diversity of wild rice. | 2005 |
| Ethiopia, Niger and South Africa | Sorghum | Determine co-occurrence of wild and domesticated sorghum, and study the potential for occurrence as weeds. | 2006 |
| Kenya and Mali | Sorghum | Identify and characterize wild sorghum populations and potential impacts of crop-to-wild gene flow. | 2004 |
| Kenya, Tanzania and Uganda | Bt cotton | Evaluate the potential for gene flow and its consequences from cultivated to wild cotton; study impacts on arthropod diversity. | 2006 |
| India | Bt eggplant | Study target pest susceptibility to Bt protein, genetic diversity using DNA fingerprinting, and parasitoids and alternate hosts. | 2004 |
| India and Indonesia | Bt crucifers | Test effect of stacked Bt genes on delaying resistance to Bt in a diamondback moth model system; study the impacts on arthropod diversity. | 2004 |
| Philippines | Bt corn | Study the direct and long-term secondary ecological effects on non-target beneficial arthropods. | 2004 |
| Philippines | Bt corn | Monitor Asian Corn Borer resistance and the impact of pollen dispersal on non-target lepidoptera. | 2005 |
| Philippines | HT corn | Use an ecosystems approach to assess potential impact of herbicide tolerant (HT) corn on wild biodiversity. | 2005 |
| Vietnam | Bt rice | Examine the impacts on the lepidopteran – based food webs in wild rice. | 2006 |



Can Bt-Cowpea be Deployed in Africa without Incidence on Wild Cowpea Biodiversity and Demography?

Year awarded: 2005

Investigators

R.S. Pasquet, African Insect Science for Food and Health, Nairobi, Kenya

J.T. Ouedraogo, Center for Environmental Research and Training (CREAF) de Kamboinse, Environment and Agricultural Research Institute (INERA), Burkina Faso

S. Demissew, Addis Ababa University, Ethiopia

P. Gepts, University of California, Davis, CA, USA

Objectives

- Assess the diversity of wild cowpea in the eastern part of its range, as well as the genetic structure of its populations, and assess the current genetic swamping of wild/weedy cowpea in West Africa by gene flow from domesticated types
- Identify and assess the importance of cowpea predators
- Assess whether wild cowpea populations are currently seed limited

Introduction

Cowpea is an essential legume crop in the African lowlands, especially in West Africa, as it is the main source of protein in this area. Cowpea is attacked by a wide range of insect pests, which may reduce yields to as low as five percent of potential harvests. Conventional breeding has failed to make significant progress against these pests. The legume pod borer *Maruca testulalis* is one of the main cowpea pests. It is not targeted by conventional, non-systemic insecticides, but it is susceptible to the Cry1Ab toxin. Ongoing progress in genetic transformation will create a Bt cowpea line with a high level of expression of Cry1Ab. Genetic transformation, therefore, seems to be the only way to develop insect-resistant germplasm, which could make cowpea one of the best models for demonstrating the potential benefits of genetic engineering in developing countries. However, cowpea has wild relatives in Africa. Research on cowpea pollinators and their efficiency shows that the Bt gene could move from cultivated to wild plants through pollen, and that the wild plants introgressed with the Bt gene could produce more seeds and become more competitive. The goal of the project is to evaluate the potential for selection for the Bt gene in wild plants to alter the genetic diversity of the wild cowpea gene pool and the plant communities where wild cowpea is growing, or cause them to become serious weeds.

Method

First, this study considers the hypothesis that wild cowpea in West Africa are weeds already genetically swamped by domesticated genes, including domesticated chloroplast. It investigates whether or not future additional swamping by genes from Bt cowpea in this area will modify drastically the biodiversity of the West African wild and weedy cowpea. As there are numerous cowpea samples from West Africa, but not from Northeast Africa, similar samplings are being done in this region (especially in the Ethiopian lowlands) to compare accessions and population genetic structure in both areas. In addition, a mix of wild and domesticated plants, as in West Africa where farmers sometimes focus primarily on fodder, leads to substantial pollen flow from wild to domesticated plants. Farmers can harvest wild-domesticated Filial 1 (F1) seeds and use them for their next sowing. Such a process may have led to a genetic swamping of the wild populations. To test this, a trial simulating this process with a well-characterized starting point is underway.

Second, this study tests the hypothesis that wild cowpea population demography is mainly regulated by predators that will not be affected by Bt cowpea, such as insects not affected by the Bt toxin, and large mammals, birds, and rodents. The presence of pre-dispersal seed predators is being recorded in wild populations, and the efficiency of the different post-dispersal predators is being assessed.

Third, the study considers the hypothesis that an increase in seed production will not lead to a proportional increase in plant demography, since seed predation may be density dependent, with the densest seed being consumed with a higher relative frequency than expected based upon its abundance. To test this hypothesis, trials based on seed addition in natural and artificial populations are being performed.



Gene Flow from Cultivated Rice (*Oryza sativa*) to its AA Genome Wild Relatives in the East African Region: Key Research for Transgene Risk Assessment

Year awarded: 2005

Investigators

S. Kiboi, School of Biological Sciences, University of Nairobi, Kenya
M. Mollel, National Plant Genetic Resources Centre, Arusha, Tanzania
T. Haileselassie, Department of Biology, Addis Ababa University, Ethiopia
D. Kiambi, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Kenya

Consultant

A. Snow, Ohio State University, Columbus, OH, USA

Objectives

- Investigate eco-geographic areas with the potential for gene flow from cultivated rice to wild rice in Tanzania, Ethiopia, and Kenya
- Investigate the frequency of crop-to-wild hybridization in experimental plots and the relative fitness of hybrid progeny from these crosses
- Investigate whether the genetic diversity of wild populations has been reduced by crop-wild hybridization and if so, whether novel transgenes might exacerbate this problem

Introduction

Worldwide, transgenic techniques are being used to enhance rice with traits for increased amounts of beta carotene (Provitamin A) and Vitamin E; high protein, iron, and zinc content; disease and insect resistance; virus resistance; salt tolerance; increased yields; and herbicide tolerance. Due to increasing demand for rice, eventual introduction of genetically modified (GM) rice in East Africa might be inevitable. At the same time, a growing demand for suitable cultivation areas is leading to encroachment on habitats where wild rice occurs. In order to lay the groundwork for the safe introduction of promising GM rice varieties in Africa, this study aims to establish possible ecological and evolutionary consequences of gene flow from cultivated rice to its wild relatives.

Although these studies do not use GM rice varieties, the findings will aid decision makers in assessing the environmental effects of all future introductions of GM rice in Africa. Basic knowledge about gene flow is needed to understand possible consequences of transgene dispersal. Furthermore, our collaborations and outreach activities will help build capacity for environmental risk assessments for other transgenic crops that may be proposed for non-regulated status in the near future.

Method

The research focuses on two native species (*Oryza longistaminata* and *O. barthii*) that occur throughout Sub-Saharan Africa and have the same AA genome as cultivated rice (*O. sativa*). These wild species grow in unmanaged wetlands and seasonal wetlands where they are viewed as valuable genetic resources for future crop breeding. They also occur in farmers' fields, where they can be troublesome weeds. The overarching questions we address are: How widely are crop genes introgressing into populations of wild rice species and over what distances? And, could specific novel transgenes enhance the weediness of these species and/or lead to erosion of their genetic diversity? To address these questions, this study investigates:

1. Eco-geographic areas with the potential for gene flow from cultivated rice to wild rice in Tanzania,

Kenya, and Ethiopia. The relative abundance of the two wild species in natural and agricultural habitats, the occurrence of putative crop–wild hybrids, observations on flowering status and proximity to cultivated rice fields, and ecological data such as insect damage, grazing pressure, and disease symptoms are being recorded. The out-crossing rates of the two species are being estimated using molecular markers (SSRs).

2. The frequency of crop-to-wild hybridization in experimental plots and the relative fitness of hybrid progeny from these crosses. Experimental plots with a cultivated rice variety that has a crop-specific phenotypic marker, and plants of the two wild species at varying distances from the cultivated variety, are being used to evaluate the frequency of crop–wild hybridization. The fitness of the new hybrids is being evaluated compared to their wild parents.

3. Whether the genetic diversity of wild populations has been reduced by crop–wild hybridization and if so, whether novel transgenes might exacerbate this problem. SSRs variation is being used to examine population genetic structure and the frequency of crop alleles in the wild species. Phenotypic genetic variation is being studied by measuring key morphological traits and standard fitness-related traits on plants from populations of both species grown in a common garden.



Continued US-Africa Research and Capacity Building on Risk Assessment of Crop-Wild Gene Flow in Sorghum

Year awarded: 2006

Investigators

A. Snow, Ohio State University, Columbus, OH, USA

T. Tesso, Ethiopian Institute of Agricultural Research, Nazareth, Ethiopia

G. Ejeta, Department of Agronomy, Purdue University, West Lafayette, IN, USA

J. Pedersen, United States Department of Agriculture- Agricultural Research Service (USDA-ARS), University of Nebraska, Lincoln, NE, USA

Objectives

- Study the genetic structure and population ecology of wild sorghum species in Ethiopia
- Determine their potential to occur as weeds in agricultural and natural habitats
- Examine whether these populations could be affected by crop-to-wild gene flow

Introduction

Sorghum is a staple food crop that originated in northeast Africa, where the diversity of wild relatives and local landraces is abundant. Ethiopia is known as a center of origin and diversity for this crop. Most sorghum in Ethiopia consists of locally produced landraces grown for local consumption. While there may be an interest in increasing sorghum yields in several African nations, for instance through the future release of GM sorghum (*Sorghum bicolor*), there is concern regarding the risks that deployment of transgenic sorghum could pose. For instance, fitness-enhancing transgenes could spread to wild populations and allow them to become more prevalent as weeds. Or transgenes and/or changing cultivation practices could threaten the survival and genetic diversity of wild sorghum populations. Given these risks, it is important to have data on the extent and consequences of gene flow from sorghum in order to make objective decisions regarding the release of GM varieties.

The long-term goals of this research are to estimate the potential for gene flow from sorghum to wild relatives in the crop's center of origin, Ethiopia; to examine possible risks associated with GM sorghum; and to develop scientific capacity through collaboration among scientists in Ethiopia and the USA. The data collected, relevant to expected GM sorghum introductions in Africa, will be published as conference proceedings and peer-reviewed papers.

Method

Populations of two groups of wild taxa are being studied—those that are common weeds in agricultural fields, and those that occur in other habitats. Standardized field surveys and common garden experiments are being conducted to collect and compare genetic and ecological data among populations and taxa.

Environmental Risk Assessment of Genetically Engineered Sorghums in Mali and Kenya

Year awarded: 2004

Investigators

F. Sagnard, S. De Villiers, and P.S. Traoré, International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), Mali and Kenya

A. Toure, Institut d'Economie Rurale du Mali (IER), Mali

B. Kanyenji, Kenya Agricultural Research Institute (KARI), Kenya

Objectives

- Identify and characterize a number of wild sorghum populations in Mali and Kenya
- Estimate crop-to-wild gene flow parameters and introgression rates at different distances and successional stages, using microsatellite DNA markers
- Characterize the habitats/ecological requirements and the demographic processes that control the dynamics of wild populations
- Test the fitness of Filial 1 (F1) and Backcross (BC) progeny from crosses between wild sorghums and disease or insect-resistant landraces

Introduction

Sorghum bicolor is the world's fifth most important cereal, with a total of 42 million hectares grown on all continents. It was domesticated in Sub-Saharan Africa more than five thousand years ago, and today represents a critical component of food security for more than 100 million people in Africa. Farmers in a large part of Africa cultivate sorghum landraces close to wild and weedy races. Sorghum may become a candidate for transformation through genetic engineering techniques, because of its economical importance for resource-poor farmers; due to the necessity for local germ plasm to rapidly adapt to global climate change; and to better fend off pest damage, such as that caused by *Striga*. This project aims to assess the environmental risks of introducing GM sorghums in Mali and Kenya, where wild and weedy relatives frequently grow close to or within farmers' fields.

The results of this project will provide new information about the potential environmental consequences of introducing GM sorghums, such as the conservation of wild agricultural diversity and weed management. The project will also contribute to the strengthening of national institutes through student and scientist training and will help build a scientific framework for environmental risk assessment studies applicable to other cultivated plants in Africa.

Method

A multidisciplinary research project is being conducted, relying mainly on *in situ* experiments together with on-station trials. This approach is particularly relevant in traditional agrosystems of Africa, where natural ecosystems and cultivated fields are highly spatially and temporally inter-connected, and where such factors as seed transports by animals and wild-type stem removal by farmers are likely to play a major role in wild population dynamics. This study is attempting to identify the morphological, genetic, and agro-ecological relationships between wild, weedy, and cultivated sorghums at the country level. In one intensive study site in Mali and Kenya, the habitats and ecological requirements of wild sorghum, and the demographic processes that control the dynamics of wild populations, are being characterized. Special attention is being paid to farmers' practices related to wild and weedy sorghum management. Using microsatellite genetic markers, estimates of the mating system and crop-to-wild/weedy gene flow parameters are being taken, as well as the introgression rate at different distances and at different successive stages of agro-ecosystem dynamics. Crosses are being made on-station between wild

sorghums and landraces known to exhibit resistance characters. The fitness of F1 hybrid and BC progenies will be tested on-station relative to wild and cultivated parents, and *in situ* relative to other grass species occurring in the same natural plant communities as wild sorghums.



Baseline Biodiversity Impact Studies of Transgenic Bt Cotton on Wild Ecosystems in the Eastern African Region

Year awarded: 2006

Investigators

W.C. Hamisy, Tropical Pesticides Research Institute (TPRI), Arusha, Tanzania
C.N. Waturu, Kenya Agricultural Research Institute (KARI), Thika, Kenya
S. Byabagambi, District Agricultural Officer, Kiboga, Uganda
R. Abdallah, Tropical Pesticides Research Institute (TPRI), Arusha, Tanzania
J.M. Pleasants and J.F. Wendel, Iowa State University, Ames, IA, USA
N.C. Stewart, University of Tennessee, Knoxville, TN, USA

Objectives

- Study and evaluate the ecological consequences of gene flow between transgenic Bt cotton and feral cotton populations and wild cotton relatives in Kenya, Uganda, and Tanzania
- Assess the impact of Bt cotton on arthropod diversity in Kenya, Uganda, and Tanzania

Introduction

Cotton is one of the primary cash crops in East Africa. It is a major source of income for farmers, as well as a raw material for industrial production. While it has high economic potential, cotton farming in the region is highly affected by a wide range of insect pests, and especially by the cotton bollworm. As a result, cotton productivity in the region has been falling annually, with yield per hectare dropping below half of the world average. To date, advances in biotechnology have enabled the development of cotton plants with genes that are resistant to insect pests. A number of varieties have been produced and are in use around the world. Reports show that Bt crops have in many cases reduced the need for pesticide applications and significantly increased yields and profits. Despite their potential, the use of biotechnology products is facing resistance in many countries due to safety concerns. Such concerns include effects on human health, damage to the natural environment, and disruption of traditional practices and economies in less-developed countries.

To address the concerns surrounding Bt cotton and enhance the technical capacity of regulatory authorities to make objective evaluations of Bt cotton applications, there is a need to build baseline biodiversity knowledge. This project will generate baseline information about the potential impacts of gene flow and impacts on non-target organisms and will also create a basis for monitoring and evaluating Bt cotton after commercialization. The results of this study will help regulatory agencies in the region evaluate trial applications, devise mechanisms for risk assessments and management, as well as raise awareness on biosafety concerns around Bt cotton in the region.

Method

The East African region is the centre of origin for the Afro-Arabian wild cotton group and includes several cotton wild relatives, as well as feral populations, of *G. barbadense*. Wild species remain a largely untapped genetic resource; examples abound of their productive inclusion in breeding programs. These native African taxa are poorly represented in collections, and information on such central taxonomic issues as natural patterns of diversity and geographic distribution is scanty. Gene flow into wild species could undermine their genetic integrity and potentially produce weed species. Gene flow into feral populations could potentially produce individuals that can out-compete native species. Therefore, one of the goals of this research is to characterize the distribution of cotton relatives in the wild and test the

possibility and consequences of hybridization and introgression. Furthermore, the possibility of hybridization and introgression to feral cotton populations is being tested.

The second goal of this project is to address concerns about the impacts of Bt cotton on non-target insects. Arthropod diversity provides stability to the productivity of the natural ecosystem in many ways. This includes natural enemies for crop pests, including the cotton bollworm in East Africa. There are concerns that the introduction of Bt cotton in agricultural ecosystems could have a number of consequences for arthropod populations, but few studies in Africa have addressed these issues. This project is considering the impact of Bt cotton on arthropod biodiversity, the effect of Bt cotton on key natural cotton enemies, and the impact of Bt cotton on non-target pests. The project is using field surveys and collection techniques in experimental plantings of Bt and non-Bt cotton.

Baseline Susceptibility and Genetic Diversity among Eggplant Shoot and Fruit Borer (ESFB) Populations

Year awarded: 2004

Investigators

S. Parimi and U.B. Zehr, Maharashtra Hybrid Seeds Company Ltd, India
B.D. Siegfried, University of Nebraska, Lincoln, NE, USA

Objectives

- Study baseline susceptibility of ESFB populations to diet-incorporated Bt protein
- Study genetic diversity and population structure of ESFB using DNA fingerprinting techniques
- Conduct observations/survey on alternate/wild hosts and parasites found on ESFB larvae

Introduction

The eggplant *Solanum melongena* is grown throughout the year in all agroclimatic zones of India and is one of the most important vegetable crops in the country. Eggplant shoot and fruit borer (ESFB) *Leucinodes orbonalis* Guenn. (Lepidoptera: Pyralidae) is recognized as eggplant's most serious economic pest, causing yield losses of up to 70 percent. India is the center of origin and diversity of eggplant. Although no information exists on ESFB in terms of origin and diversity, it has been concluded that ESFB is also native to India due to its monophagous nature on eggplant. In addition to ESFB, eggplant is also infested with two other lepidopteran pests—fruit borer (*Helicoverpa armigera* Hübner) and stem borer (*Euzophera perticella* Rag.). As pest management options are generally limited, planting Bt eggplant presents a promising choice to both target pest control and also conserve non-target and beneficial insect species. With the intent of developing effective and environmentally friendly management options for ESFB, eggplant was engineered with the Cry1Ac gene by the Maharashtra Hybrid Seeds Company Ltd. (MAHYCO), and field trials are in progress. MAHYCO is also the lead partner in the Bt eggplant project of the Agricultural Biotechnology Support Project II (ABSP II), funded by USAID.

Method

This project focuses on understanding ESFB populations prior to Bt eggplant deployment. It is considering the following three aspects:

1. At a fundamental level, the study is working to determine the baseline susceptibility of different geographical populations of ESFB prior to Bt eggplant commercialization. The baseline data generated with respect to Bt susceptibility will be helpful in arriving at a diagnostic/ discriminating dose for future resistance monitoring programs. Laboratory selections of ESFB reared on a Bt diet will determine the potential for resistance evolution. Identification of resistant strains will also be useful in assessing cross-resistance to other Bt toxins, and in identification of molecular mechanisms of resistance and potential target sites for Bt toxins.
2. The project is also attempting to understand the genetic diversity/relatedness of geographically distinct populations of ESFB at a molecular level, and is trying to determine whether the differential susceptibility responses of populations translate to significant differences at the molecular level. The bioassay, as well as molecular data generated from these studies, will directly feed into the Government of India regulatory requirements for commercial release of Bt eggplant.

3. Lastly, this study is also including a survey of alternate/wild hosts and parasites found on ESFB larvae, with their percent parasitism. The survey will provide baseline information for subsequent research that might be conducted to assess the impact of Bt eggplant on the abundance of ESFB and insects that depend upon this species in India. The genetic diversity from molecular studies, information on alternate hosts grown in the vicinity of eggplant grown fields, and information on parasitoids will help in planning Bt eggplant deployment with a definitive refuge strategy. Information generated from this study, along with the existing knowledge of the biology and behavior of insects, and information on alternate hosts will also be important to the development of simulation models that predict the possible development of Bt resistance in the field and define resistance management strategies prior to commercialization.



Risk Assessment and Management Options for Stacked-Gene Transgenic Crucifers in India and Indonesia

Year awarded: 2004

Investigators

A.M. Shelton, Cornell University/ New York State Agricultural Experiment Station (NYSAES), Geneva, NY, USA

R.T. Roush, University of California, Davis, CA, USA

D.A. Russell, LaTrobe University, Victoria, Australia

G.T. Gujar, Indian Agricultural Research Institute, New Delhi, India

A. Rauf, Institut Pertanian Bogor (IPB), Bogor Agricultural University, Bogor, Indonesia

Objectives

- Test the efficacy of this dual gene system in delaying the development of resistance to Bt in diamondback moth (DBM), using Bt plants and/or simulated Bt spray formulations
- Compare the relative impact on selected target and non-target arthropods of Bt transgenic crucifers, foliar Bt products, and the most commonly used synthetic insecticide

Introduction

Vegetables in the crucifer family (cabbages, cauliflower, broccoli, etc.) are important components of diets in India, Indonesia, and throughout Asia. Crucifers have many insect pests, but by far the most damaging is the diamondback moth (DBM), *Plutella xylostella*, with global annual management costs estimated at US\$1 billion. Control of DBM through the use of conventional insecticides can result in environmental and human health concerns. An alternative approach to control DBM is to use crucifer plants that have been engineered to express proteins from the bacterium, *Bacillus thuringiensis* (Bt). Bt maize and cotton plants are already used on 18 million hectares worldwide. This project will help provide regulators in India and Indonesia with the necessary in-country expertise to make informed decisions about registering Bt crucifers and will also provide additional information about the general strategy of using pyramided Bt toxins for sustainable insect control.

Method

A public/private partnership is being built to provide stacked-gene Bt brassicas for insect control in the developing world. The first releases are planned for India and Indonesia after global and local regulatory requirements are met. Because of the potential for DBM resistance evolution to Bt plants, this project uses pyramided (i.e. dual) Bt toxins that do not share a common target site. Models and studies at Cornell University have shown this to be an effective strategy for delaying resistance.

The dual gene Bt brassica system is being used by scientists from Indonesia, India, and the US to develop risk assessment techniques on the effects of the Bt plants on pest management of the caterpillar complex, other insect pests, and non-target beneficial arthropods. Cooperatively, methods are being developed and tested to compare the relative impact on selected target and non-target arthropods of Bt transgenic crucifers, foliar Bt products, and the most commonly used synthetic insecticide. The frequency and inheritance patterns of genes conferring resistance to the proposed Bt proteins in DBM in major crucifer production areas in India and Indonesia are also being examined, and ways in which resistance can be delayed are being explored.

Investigation of Secondary Ecological Effects of Bt Corn in the Philippines

Year awarded: 2004

Investigators

E. Alcantara, National Institute of Molecular Biology and Biotechnology, University of the Philippines, Los Baños, Philippines

B. Lavina-Caoili, Department of Entomology, University of the Philippines, Los Baños, Philippines

P.A. Javier, National Crop Protection Center, University of the Philippines, Los Baños, Philippines

M. Dulce J. Mostoles, Camarines Sur State Agricultural College, Pili, Philippines

Objectives

- Study the direct effect on indicator species using the maximum hazard dose approach
- Study the long-term effect on indicator species using exposure characterization in the greenhouse
- Conduct on-farm studies of indicator species abundance and species composition
- Study the use of riparian areas by indicator species

Introduction

Commercial-scale propagation of Bt corn in the Philippines was approved by the government in 2002. Since then, the Philippines has become one of the leading countries in the global adoption of transgenic agriculture. Bt corn, expressing the transgenic insecticidal Cry1Ab protein, works to control the Asian corn borer (ACB), *Ostrinia furnacalis*, the major insect pest of corn in the Philippines. This research project tests the following hypotheses:

- 1) Cry1Ab protein is not toxic to *Micraspis discolor* and *Orius tantillus*;
- 2) Bt corn does not cause adverse secondary ecological effects in corn and riparian ecosystems, such as a significant reduction in the abundance of indicator insect species; and
- 3) Bt corn does not trigger insect–pest succession.

Method

Good indicator species in the local corn ecosystem are the coccinellid beetle *Micraspis discolor* and the anthocorid bug *Orius tantillus*, which are both natural predators of ACB. Tier 1 testing using the maximum hazard dose was conducted to determine acute toxicity of Cry1Ab protein to *M. discolor* and *O. tantillus*. Long-term (chronic toxicity) effects on ecological fitness (fecundity, predation rate, longevity) were not determined because of the absence of acute toxicity. Now, on-farm studies are being conducted to determine the effect of Bt corn on *M. discolor* and *O. tantillus* abundance and to monitor shifts in species composition that might be indicative of pest succession.

In addition, to determine the use of riparian areas (land immediately surrounding a water source) by indicator species, the relationship of the Bt corn ecosystem to the natural biodiversity under tropical conditions is being investigated. Riparian areas can be found in corn-growing regions of the country. Because of favorable tropical conditions, these areas are important to consider as they not only support high levels of biodiversity, but also contribute to the overall stability of ecosystems. It might be possible that a species could use a riparian area as a refuge or as a link to travel between two ecosystems. It is therefore useful to discover if adverse secondary ecological effects also occur in the riparian area as a result of natural biodiversity exposure to Cry1Ab protein carried by *M. discolor* and *O. tantillus* from Bt corn.

Outcomes

Results of laboratory feeding experiments revealed that Cry1Ab protein has no acute toxicity to either *M.*

discolor or *O. tantillus*. Arthropod abundance data were also gathered from experimental corn fields and riparian sites. Repeated measures analysis of variance (RM-ANOVA) showed that there is no significant difference in the abundance of *M. discolor* in either the Bt corn, non-Bt corn, or in the riparian areas. The peak abundance of *M. discolor* in both Bt and non-Bt corn is observed during the reproductive silking stage. The field study did not detect a significant treatment effect in any of the experimental sites with regard to arthropod guild composition, ecological function (represented by the unweighted predator/prey ratio), and arthropod diversity. Results of principal response curve analysis showed no adverse change in the community arthropod species composition in all experimental sites.

Taken together, the laboratory and field data support the hypothesis that Bt corn has no acute toxicity to *M. discolor* and *O. tantillus* and that the deployment of Bt corn in the field will not significantly diminish the abundance of these predators in a way that will cause damage to the stability of arthropod communities in the corn and riparian ecosystems. Correspondence analysis of abundance data does not show an indication of pest succession.

Impact

The data generated from laboratory and field experiments were used by the Bureau of Plant Industry in early 2008 as a basis for renewing the license for commercialization of Bt corn Mon810 YieldGard in the Philippines.

Post Commercialization Monitoring of Asian Corn Borer (ACB) *Ostrinia furnacalis* Resistance to Bt Corn in the Philippines and the Impact of Pollen Dispersal on Non-target Lepidoptera

Year awarded: 2005

Investigators

B.F. Cayabyab, College of Agriculture, University of the Philippines, Los Baños, Philippines
E.P. Alcantara, BIOTECH, University of the Philippines, Los Baños, Philippines
A.C. Sumalde, Department of Entomology, University of the Philippines, Los Baños, Philippines
W.R. Cuaterno, Crop Protection Division, Bureau of Plant Industry, San Andres, Manila, Philippines
B.D. Siegfried, University of Nebraska, Lincoln, NE, USA
P. G. Gonzales, College of Agriculture, University of the Philippines, Los Baños, Philippines
J.M.M. Belen, College of Agriculture, University of the Philippines, Los Baños, Philippines
J.G.P. Dangan, College of Agriculture, University of the Philippines, Los Baños, Philippines
M. C.T. Malenab, College of Agriculture, University of the Philippines, Los Baños, Philippines

Objectives

- Determine the initial frequency of Cry1Ab resistance alleles in ACB
- Determine the number of receptor classes in different populations of ACB
- Clone the ACB receptor and study its Cry1Ab binding kinetics
- Measure the extent of dispersal and density of Bt corn pollen deposition on the host plant of the nontarget lepidopteran *H. bolina*
- Determine the susceptibility of the nontarget lepidopteran *H. bolina* to Cry1Ab protein

Introduction

Since the government of the Philippines approved the genetically modified crop, Bt corn, for planting and commercial use in 2002, farmer adoption of Bt corn has been steadily increasing. To date, an area of more than 400,000 hectares has been planted. Post-commercialization monitoring of the crop's performance is necessary in order to detect, as early as possible, the potential development of Asian Corn Borer (ACB) resistance and any possible effects of Bt corn on native biodiversity.

The following studies will estimate the frequency of Cry1Ab resistance alleles in ACB to serve as quantitative baseline data for monitoring resistance in ACB. Also, they will evaluate the nontarget effects of Bt corn on the butterfly *Hypolimnas bolina* using experiments designed to a) determine level and route of exposure to Bt corn, b) delineate hazards, and c) assess if the hazards constitute a risk, in this case, on biodiversity.

Method

Study 1. F2 Screen. This study tests the hypothesis that the frequency of Cry1Ab resistance in ACB is low ($<10^{-3}$). The method of Andow et al. (1998) is used to search for resistance alleles in ACB. The progeny (F1) from each field-collected female are sib-mated within each family line and reared on an artificial corn borer diet. The succeeding neonate larvae (F2) are assayed in an artificial corn borer diet containing a discriminating dose ($LC99 = 120 \text{ ng/cm}^2$) (Alcantara et al., unpubl.) of Cry1Ab protein. The results are analyzed using Bayesian statistics as described by Andow and Alstad (1998).

Study 2. Cry1Ab receptor protein profile of different ACB populations and identification of putative receptor genes. This study tests the hypothesis that Cry1Ab binding to the midgut receptor is similar among the different ACB populations and is highly related to European corn borer, *Ostrinia nubilalis*.

The receptor cloning and binding kinetic studies should stimulate further research on the molecular basis of insecticidal action and ACB resistance to Cry1Ab protein. This study is being conducted at the laboratory of Dr. Blari Siegfried at the University of Nebraska, Lincoln. The corn borers come from the Philippines.

Study 3. Bioassay of Cry1Ab protein to non-target Lepidoptera. This study aims to determine if the ingestion of Cry1Ab protein is a hazard to *H. bolina* butterfly larvae. *H. bolina* is not a pest of corn but is a cosmopolitan butterfly, and a good indicator of biodiversity. This butterfly is commonly observed in corn fields and adjacent areas, and so is likely exposed to Bt corn pollen in the field. Bioassays using the maximum hazard dose of Cry1Ab protein, at 10 times the maximum concentration in Bt corn, are currently being conducted.

Study 4. Effect of Bt corn pollen on the survival of non-target Lepidoptera. This study focuses on larval exposure to pollen deposited on *Ipomoea triloba* leaves as a source of risk of intoxication. *I. triloba* is a host plant of *H. bolina* and is a common weed in corn fields. This study is composed of three parts:

- 1) The first part attempts to determine the actual exposure level of *H. bolina* larvae to Bt corn pollen.
- 2) The second part attempts to determine adverse effects on *H. bolina* larvae from actual field exposure to Bt corn pollen by conducting pollen bioassays.
- 3) The last part involves absolute larval sampling to determine the proportion of *H. bolina* populations that are actually exposed to Bt corn pollen. It aims to determine if Bt corn pollen sheds coincide with the presence of *H. bolina* larval populations.

Ecosystems Approach in the Assessment of the Impacts of Herbicide-tolerant (HT) Corn on Wild Biodiversity in Selected Areas in Luzon, Philippines

Year awarded: 2005

Investigators

C.I. Villamor, V.O. Sinohin, S.A. Pasicolan, E.F. Rimando, C.C Marquez, C.G. Taguiam, P.C.Atega, and C.M. Palaypayon, Ecosystems Research and Development Bureau (ERDB), Department of Environment and Natural Resources (DENR), College, Laguna, Philippines

Objectives

- Assess and compare the dynamics of plant succession in herbicide-tolerant (HT) corn fields, conventional farm areas, and adjacent marginal areas or forest edge, taking into consideration the type of corn production system
- Assess and monitor spatial and temporal changes in avifauna associated with corn
- Assess HT corn's effects on arthropods in relation to the changes in plant species composition
- Assess and compare the soil characteristics associated with the use of conventional and HT corn

Introduction

Advances in Philippine modern biotechnology have progressed so that it is now possible to monitor environmental impacts following the commercial-scale introduction of genetically engineered crop plants. Herbicide-tolerant Roundup Ready®, corn, commercialized in April 2005, was viewed as a suitable alternative to the traditionally laborious and expensive hand weeding practices that farmers employed in an effort to avoid excessive losses in corn yield. The promise of the technology is based on reducing herbicide use in post-emergence situations, using the broad-spectrum herbicide glyphosate, which breaks down rapidly in the soil. In this project, the potential impacts of HT corn on Philippine wild biodiversity are viewed in the context of the potential changes in weed composition and population, and subsequent impacts on insects and resident avifauna that are normally associated with agricultural areas.

The implementation of this three-year project is premised on the following hypotheses:

- 1) Adopting the technology package for HT corn could result in weed shifts and reduce weed populations. Such effects might be more pronounced when HT corn is grown continuously throughout the year, rather than when one crop of corn is grown alternately with another crop (negative impact);
- 2) A decline in weed populations and seed abundance could cause a decline in avian fauna, particularly the seed- and insect-eaters commonly associated with agriculture, leading to a decline in wild biodiversity (negative impact); and
- 3) Adopting minimum tillage practices as prescribed in the technology package for HT corn could improve soil conditions and reduce soil erosion, which could possibly improve crop yield (positive impact).

Method

The project considers the corn–corn production system as practiced in Camarines Sur (Region 5) and the rice–corn production system in Pampanga (Region 3). The experimental sites are located close to known centers of biodiversity, namely, Mt. Isarog National Park and Mt. Arayat National Park, respectively. A total of five hectares each of conventional hybrid corn (control) and HT corn (experimental treatment) were established during wet season planting (May to June) in 2006 in Camarines Sur. This represented the corn–corn production system by partnering and sharing resources with local farmers in the area. A similar experimental set-up was also adopted in Pampanga, with dry season corn planting starting in December 2006.

This study is designed to cover a routine monitoring of data through at least six growing seasons of corn in Camarines Sur and two seasons of corn grown alternately with three seasons of rice in Pampanga, including:

- 1) Assessing the changes in weed species composition and diversity during specific stages of the corn-growing season and fallow period for the two types of corn production systems;
- 2) Routine monitoring of both above- and below-ground arthropods and other beneficial insects;
- 3) Monitoring of avifaunal composition diversity with emphasis on seed- and insect-eaters;
- 4) Assessing physical changes in soil characteristics, principally soil fertility and erosion rates; and
- 5) Analyzing and identifying other sociological attributes that influence the adoption of HT corn and other varieties of corn in the area.

Modeling the impacts of Bt transgene flow on lepidopteran food web structure and stability on wild rice in Vietnam

Year awarded: 2006

Investigators

N.L. Cuong, Cuu Long Rice Research Institute, Can Tho, Vietnam
Y.H. Chen, University of Vermont, Burlington, VT, USA
A.T. Barrion, PhilRice Los Baños, College, Laguna, Philippines
K.L. Heong, International Rice Research Institute (IRRI), Manila, Philippines
G. Langellotto, Fordham University, Armonk, NY, USA
B. Lu, Fudan University, Shanghai, China
N.V. Tuat, Food Crops Research Institute, Vietnam

Objectives

- Document the diversity and abundance of non-target lepidoptera and their natural enemies in wild rice ecosystems
- Assess variation in non-target lepidopteran susceptibility to Bt crop/wild hybrids
- Determine the structure of a model lepidopteran-based food web
- Characterize lepidopteran-based food webs to predict how the Bt gene may impact arthropod food webs

Introduction

Rice is a critical food crop in Vietnam, with over seven million hectares of cultivation and around 36 million tons of total production. Biotechnology applications such as transgenic Bt rice could play a significant role in ensuring national food security and alleviating poverty, but Bt rice may also pose important environmental risks. Therefore, environmental biosafety assessments are needed prior to the commercialization of any transgenic rice. This project will address the consequences of gene flow from transgenic rice on non-target arthropods in wild rice ecosystems.

Transgenic rice with the Bt toxin is currently being developed and evaluated in Vietnam. However, there are important biosafety concerns regarding Bt rice release. The release of transgenic rice would occur in the region of crop origin, where wild ancestors are found adjacent to cultivated rice throughout the Mekong Delta. Therefore, regulations mandating minimum planting distances from wild relatives may be difficult to enforce. Furthermore, although the Bt toxin in the transgenic rice targets lepidopteran pests, non-target lepidopteran insects may also be susceptible. Therefore, if the Bt gene enters and is fixed in *O. rufipogon* populations, Bt rice might impact native lepidoptera and arthropod community structure. If the Bt transgene becomes established after hybridization, there may be effects on arthropod diversity, food web structure, and food web stability. Vietnam is currently drafting its biosafety regulations. Therefore, the results of this project will provide timely and relevant information for the regulatory framework regarding the biosafety and sustainability of Bt rice in Vietnam.

Method

Empirical and theoretical approaches predict the impact of escaped genes from Bt rice on arthropod biodiversity in wild rice ecosystems, including: 1) Monitoring field populations to document the diversity and abundance of non-target lepidoptera and their natural enemies in wild rice ecosystems; 2) Using feeding studies to assess variation in non-target lepidopteran susceptibility to Bt crop/wild hybrids; 3) Using stable isotope analysis to identify trophic positions to determine the structure of a model lepidopteran-based food web using; and 4) Using a caged mesocosm experiment to characterize lepidopteran-based food webs to predict how the Bt gene may impact arthropod food webs.

Program for Biosafety Systems

International Food Policy Research Institute
2033 K Street, NW, Washington, DC 20006 USA
T: 202.862.5600 F: 202.467.4439 E: ifpri-pbs@cgiar.org
www.ifpri.org/pbs/pbs.asp