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ADVANCES IN DEVELOPING INSECT RESISTANT MAIZE VARIETIES FOR KENYA WITHIN THE INSECT RESISTANT MAIZE FOR AFRICA (IRMA) PROJECT

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ABSTRACT

Lepidopteran stem borers are economically important pests of maize, a major staple in Kenya. The Insect Resistant Maize for Africa (IRMA) Project aims at increasing maize production and food security through the development and deployment of insect resistant maize. Bt maize utilizes genes that encode delta-endotoxins; proteins derived from the soil bacterium *Bacillus thuringiensis* (Bt). Suitable genes have been acquired or synthesized and backcrossed into elite maize germplasm. Clean Bt gene events containing only the gene of interest and no antibiotic or herbicide resistance markers have been developed. Bt maize leaves have been introduced into Kenya, insect bioassays carried out and the effective cry proteins against major maize stem borers identified. Insect resistant maize varieties are being developed using conventional breeding and locally adapted as well as exotic germplasm. To ensure safe dissemination of Bt maize, studies on their impacts on target and non-target arthropods mainly through their characterization and quantification have been done. Insect resistance management strategies are being developed and studies on gene flow are under way. Impact studies have revealed factors in the society that will hinder or enhance adoption of Bt maize as well as establish baseline data that will form the basis of monitoring and evaluation. Technology transfer and capacity building, creating awareness and communications are important activities in the project. These activities address the various concerns that surround the use of genetically modified organisms. This paper provides a general overview of the IRMA project, presents brief results from various activities and examines how the various concerns about GMOs are addressed within the project activities.

INTRODUCTION

Stem borers are the most widely distributed and most damaging pests to maize worldwide. In Africa, there are several economically important stem borer species. The most important borers are the spotted stem borer (*Chilo partellus* Swinhoe) and the African stem borer (*Busseola fusca* Fuller). Lepidopteran stem borers are economically important pests of maize, a major staple in Kenya (Seshu Reddy and Sum, 1991). *Chilo partellus* is found mainly in East Kenya, and is the most destructive pest of maize in warm, low-altitude regions. *Busseola fusca is* mainly in West Kenya, is native to Africa and is the major borer pest in the highlands. Host plant resistance developed through conventional breeding methods and through genetic engineering, especially Bt maize, has potential to help resource-poor farmers combat stem borer damage. Bt maize, a genetically modified organism (GMO), utilizes genes that encode delta-endotoxins; proteins derived from the soil bacterium *Bacillus thuringiensis* (Bt).

The Insect Resistant Maize for Africa (IRMA) Project is a joint project between Kenya Agricultural Research Institute (KARI) and the International Maize and Wheat Improvement Center (CIMMYT) with financial support from the Syngenta Foundation for Sustainable Development (KARI and CIMMYT, 2001). The goal of the IRMA Project is to increase maize production and food security through the development and deployment of insect resistant maize to reduce losses due to the stem borers, first in Kenya, and later in other African Countries. The IRMA project focuses on identifying the best technologies, or combination of technologies to combat stem borers, developed conventionally or through biotechnology for African farmers. The major objectives of the project are to develop insect resistant maize varieties for the major Kenyan maize growing environments, and to establish procedures to provide insect resistant maize to resource-poor farmers in Kenya

Genetically modified organisms (GMOs) hold much potential in enhancing food production through technologies that lead to reduced input use, reduced risk from biotic and abiotic stresses, increased yields, and enhanced quality of agricultural products (Mann, 1999). GMO technology has seen rapid adoption in industrial countries reaching 35 million hectares in the year 2000, with a slower but steady rate of adoption in developing countries, reaching 10 million hectares in the year 2000 (James, 2001). Most developing countries are beginning to evaluate the potential of such technologies for meeting their food security needs.

GMO technology has been debated due to various concerns regarding its use, such as risks to human health, ecological and environmental risks, and build up of resistance by target insects. Developing, testing and disseminating insect resistant maize varieties involving Bt technology, therefore, required different approaches from those employed when using conventional methods. Such approaches have to consider effects on the human communities and the environment. The IRMA project, therefore, is comprised of activities that include: product development (Bt genes and maize varieties), product dissemination (ecology and insect resistance management), impact assessment, technology transfer, and awareness creation and communications. This paper provides a general overview of the IRMA project, presents brief results from impact assessment, product development, insect resistance management and communications activities during the first two years, and describes how the various concerns about GMOs are addressed within the project activities.

RESEARCH ACTIVITIES OF THE IRMA PROJECT

Product Development – Bt genes and Bt maize source germplasm:

Bt-maize involved the first transgenes to be handled by CIMMYT and its development is viewed as a valuable component in meeting the food security needs of clients in developing countries. CIMMYT has acquired Bt-maize events from the private sector and public sector as well as synthesizing other Bt genes with partners. Research Bt maize has been enhanced and legislation in Mexico has been instituted. Some CIMMYT lines were converted through conventional backcrossing to generate backcrossed lines containing *cryIAb*. CIMMYT has produced transformed plants that show integration of the *cryIAc*, *cryIB* and *cryIE* with*,* maize ubiquitin and rice actin promoters.

Specifically, various *Bt cry* genes (*cry1Ab, cry1Ac, cry1B, cry1E, and cry B-1Ab*) were used to successfully transform the CML216 x CML72 hybrid maize. Backcrosses were made to CML216 and the lines (T0 - T4) have shown high levels of resistance to stem borers (Table 1). Recently, development continued on second generation events that carry only the gene of interest. These "clean genes" do not carry the selectable Basta herbicide resistance (the *bar* gene) marker, and so bypass potential risks raised by some about the technology (KARI and CIMMYT, 2001). These events are developed by using isolated *Bt* and *bar* gene sequences for transformation. In addition, the *Bt* and *bar* genes are co-transformed, and they will be inserted separately into the maize genome. This increases the possibility of separating the two genes in the final product, thus producing an insect resistant, but herbicide susceptible variety. This approach is critical in ensuring that concerns associated with Bt maize are addressed. The various events have now been characterized for molecular composition.

Table 1. First generation transgenic Bt maize..

Event	Genes introduced	Generation
E176	PEP:*cry*IAb-Pol:*cry*IAb + 35S:*bar*	T16+ (BC16+)
E5207	Ubi:*cry*IAc + 35S:*bar*-35S:gus	T4
E5601	Act:*cry*IB-35S:*bar*	T4
E1835	Ubi:*cry*IB-35S:*bar* + 35S:*bar*	T2
E602	Act:*cry*IE-35S:*bar*	T2
E7	Ubi:*cry*IB-Iab fusion-35S:*bar*	T2

One of the highest priorities is the identification of which Bt genes are most effective against each of the targeted insect pests. There are several methods to determine the activity of Bt genes such as insect bioassays using isolated Bt proteins or immunological assays of labelled Bt proteins against isolated insect mid-guts (to determine whether receptors are present in the mid-gut). In our experience, the protein bioassays are easy but often do not indicate the most effective proteins. The immunological tests are highly accurate but are technically challenging and require special expertise and infrastructure. Ultimately, the best assay is the effect the Bt maize plants have against insects. Given the early state of biosafety in Kenya and the lack of proper infrastructure in KARI to handle transgenic maize (in the lab and the field), we decided that the simplest procedure would be to import Bt maize leaves (that were grown in CIMMYT's biosafety greenhouses in Mexico) into Kenya and perform leaf bioassays in the KARI-NARL Biotechnology Laboratories.

To introduce the leaves from Bt maize into Kenya, a permit was issued by the Kenya National Biosafety Committee (Mugo *et al*., 2002, this volume). Bioassays were carried out to identify the effective Bt genes against five Kenyan stem borers. The *cry*1Ab protein was the most active against all species as shown by the least area of leaves consumed and by the low percentage of larvae that were killed. *Chilo partellus* was affected by all cry proteins, except *cry*1E. *Eldana saccharina* was the least affected by any cry protein. *Chilo orichalcocilielus* was most affected by cry1Ab and cry1B proteins. *Sesamia calamistis* was affected by *cry*1Ab and *cry*1Ab-1B proteins. *Cry*1E protein was not active against any species. The tested Bt cry proteins were not effective in the control of *Busseola fusca*. We may need a combination of cry proteins being expressed at high levels or other Bt cry proteins like *cry*IC to effectively control *B. Fusca*. A prospective control was identified for *Chilo partellus,* the most destructive and most widely distributed stem borer in Kenya.

These results will be verified under biocontainment greenhouses and open quarantine field site facilities that are being developed currently. A field site has been developed at Kiboko within the KARI-NRRC Kiboko, which will be isolated by distance planting. No maize will be grown within 200m of the one-hectare chain-link fenced field. Maize will be detasselled to prevent inadvertently effecting gene flow to the maize crop and any other plant species. Tests will be done by infesting maize containing various *cry* genes singularly and in combinations with *Chilo partellus* and *Busseola fusca* stem borers. This information will allow the better targeting of the development of Kenyan maize varieties with the appropriate combinations of genes for resistance to these stem borer species.

Product Development – Locally adapted non-transgenic and transgenic insect resistant maize germplasm:

Host plant resistance is an approach to stem borer control, by which the plant itself is resistant to the stem borers. Host plant resistance is transferred to farmers in the seed, a fact that ensures that the technology is inexpensive, safe, and that the farmers need not purchase more inputs to control stem borers. Use of stem borer resistant maize increases efficiency of farming by reducing or eliminating the expense of insecticides and reducing yield losses from stem borer damage. For resource-poor small-scale farmers in developing countries, therefore, host plant resistance packaged into improved varieties will offer a practical and economic means of minimizing stem borer losses.

The IRMA project is primarily focusing on developing stem borer resistant maize varieties, an activity that falls into two categories: 1) search for sources of resistance and development of source germplasm for insect resistance, and, 2) search for elite germplasm to backcross to Bt genes sources when these will be available. The development of source germplasm is based on utilizing genes and sources of resistance already existing in the maize plant. We have evaluated 216 Genotypes from CIMMYT and KARI, 42

MBR S4 lines from CIMMYT Mexico, and 500 inbred lines from Mexico. In the search for stem borer resistant elite germplasm, 330 maize OPVs and hybrids have been evaluated in different maize growing ecologies in Kenya. This germplasm has been evaluated for resistance to *Chilo partellus* and *Busseola fusca* stem borers through artificial infestation. The germplasm is also being screened for tolerance to local stresses such as drought and low nitrogen, resistance to maize streak virus, *Turcicum* blight, leaf rust, and weevils in storage to ensure that insect resistance will be in good adapted genetic backgrounds. Good stem borer resistant inbred lines are being crossed to heterotic testers like CML78 and CML444, while combining ability studies are being done to identify lines with good specific and general combining abilities. Suitable hybrids will be made from lines with good specific combining abilities, while synthetics will be developed from good lines with good general combining ability. Recycling of inbred lines is being done to develop elite locally adapted germplasm. We are identifying good sources, especially those carrying resistance to more than one stem borer species.

Product Dissemination – Potential effects of Bt maize on non-target arthropods.

Knowledge of the environmental impacts of Bt-gene based stem borer resistance technology on non-target organisms in the major maize cropping systems is essential for the safe deployment of *Bt*-maize. Studies have been made to identify and determine the relative abundance of the target and non-target arthropods of *Bt*-maize in major maize growing regions in Kenya (Songa *et al*., 2001, this volume.). A reference collection of arthropods has been established, that will serve as a technical reference during the monitoring of effects of Bt maize later in the IRMA project cycle. Arthropod characterization studies were conducted in three agro-ecologies: i) lowland tropics (Kilifi district), ii) dry mid-altitude (Machakos district), and iii) moist transitional (Kakamega). Different–sampling methods were used for the various groups of arthropods. For soil crawling arthropods, pit-fall traps were used. For flying arthropods, two types of traps were used: water traps using yellow basins positioned at 1.2m above ground, and sticky traps using clear glass painted with insect glue and positioned at 1.2m above ground. For soil crawling arthropods, pit-fall traps were used. In each farm, 50 randomly selected plants were inspected for stem borers and other arthropods, at each of three plant growth stages: mid-vegetative, reproductive (tasseling and silking), and maturity stages (Oloo, 1989).

Among the target organisms, the stem borers that infested farmers' maize fields were identified in each of the sites in descending order of abundance as: Kilifi - *Chilo partellus*, *Chilo orichalcociliellus*, *Sesamia calamistis*, and *Cryptophlebia leucotreta*; Kakamega – *Busseola fusca*, *C. partellus*, *S. calamistis* and *C. leucotreta*; Machakos – *C. partellus*, *S. calamistis* and *C. leucotreta*. This suggests that, in order to have an impact on stem borer damage in maize, pest management technologies (e.g. *Bt*-maize), should be targeted at each of these key stem borer species in the respective regions (Songa *et al*., 2001).

Among non-target organisms,: Most of the parasitoids of stem borers recovered in each of the study sites were the larval type, with Kilifi having the widest diversity of parasitoids (6 species), followed by Machakos (3 species) while only two species were found in Kakamega. The exotic larval endoparasitoid *C. flavipes* was recovered from Kilifi and Machakos where releases were made in 1993-1997 (Overholt *et al*., 1997), which shows good establishment and spread and the need to study the non-target effects of *Bt*-maize on *C. flavipes*.

Among arthropods, the diversity of arthropod families recovered from traps in the different maize cropping systems was 69, 67 and 59 species in Kilifi, Kakamega and Machakos, respectively. Out of the wide range of arthropods recovered, five categories of non-target arthropods of interest have been identified, including the potential biological control agents, pollinators, decomposers of organic material in the soil as the most abundant ones. Some arthropods were abundant in all the three study sites, while some were limited to specific sites.

The arthropods that were most frequently recovered from the maize plants in Kakamega, Kilifi and Machakos were Formicidae, Forficulidae, Blattidae and Araneida. Formicidae (ants) and Forficulidae (earwigs) are known to be predators of stem borer eggs and larvae (Oloo, 1989). Ladybird beetles, which are known to be predators of *C. partellus* eggs (Dwumfour et al., 1991), were also recovered, with *Cheilomenes sulphurea* (Olivier) being the most common species, especially at Kakamega

All pest management technologies will have effects on organisms dependent on the target pests for food. One objective of development of insect resistant genetically modified crops is to reduce the reliance on conventional broad-spectrum insecticides (Morton *et al*., 2000). The environmental impacts of using transgenic crops such as *Bt*-maize have therefore to be evaluated and judged alongside the commonly used conventional insecticides (Hails, 2000). Before the deployment of *Bt*-maize into Kenya, it would be useful to standardize the protocols to be used in evaluating the impacts of *Bt*-maize alongside the conventional insecticides.

A study was conducted to evaluate Thuricide, a *Bt*-biopesticide and Dimethoate, conventional insecticides in the management of stem borers and the effects on non-target arthropods, in a maize/bean cropping system to evaluate their control of stem borers in maize, their effects on non-target arthropods in a maize bean cropping system, and to standardize the protocols to be used in evaluating the environmental impacts of *Bt*-maize alongside conventional insecticides in the field

Three types of traps were used: pit-fall, sticky and water traps. The stem borers that infested maize in the insecticide trial at Katumani field station, in descending order of abundance, were: *C. partellus* (65.8%), *S. calamistis* (21.4%), *C. leucotreta* (8.3%) and *B. fusca* (4.5%). The level of stem borer infestation was lowest in the *Bt*-sprayed plots (0.019 borers per plant) followed by the conventional insecticide (CI) treated maize plots (1.02), while the untreated maize had the highest stem borer intensity (3.41 borers per plant). The *Bt*- sprayed maize had significantly lower stem borer damage, in terms of the number of moth exit holes, tunnelling length and the percentage of damaged plants, compared to the CI and the control (untreated) maize (Table 2). Although the CI maize had a significantly lower number of stem borer exit holes and tunnelling length than the untreated maize, the percentage of infested plants was similar in these two treatments.

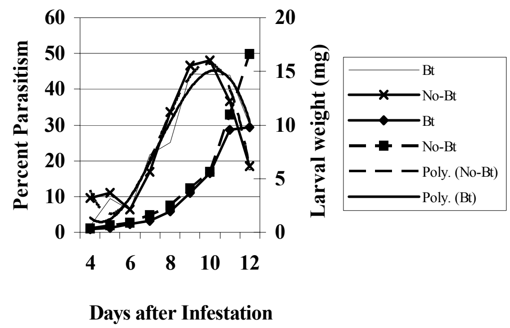
Five different parasitoids were recovered from the three treatments, although the parasitism levels were generally quite low. The untreated plot had the widest diversity of

Table 2. Mean number of stem borer exit holes, tunnel length and the percentage of maize plants infested by stem borers, in each of three treatments in Katumani.

Treatment	Exit holes*	Tunnel length (cm)*	Percent infestation**
Dimethoate	2.70 ± 0.39 b	3.93 ± 0.73 b	59.04 ± 1.66 a
Thuricide[#]	0.80 ± 0.16 c	1.80 ± 0.44 c	23.21 ± 3.34 b
Control	6.97 ± 0.90 a	12.70 ± 1.88 a	72.37 ± 6.17 a

Thuricide[#] - *Bt*-spray;
Values followed by the same lower case letter within the same column are not significantly different (Tukey's test, P>0.05). Exit holes: F=43.09; df=2,177; P=0.00;
Tunnel length: F=28.64; df=2,177; P=0.00; Percent infestation: F=26.36; df=2,6; P=0.001
*/** - Data analysis was on log (x+1) and arcsin (x/100) transformations respectively, but the values presented are untransformed.

Figure 1. Parasitism of a wasp (*Campoletis sonorensis*) on fall armyworm (*Spodoptera frugiperda*) in Bt and non-Bt maize.



parasitoids and these were *C. flavipes* (6.4%), *C. sesamiae* (0.97%) and *P. furvus* (3.1%). Only two species of parasitoids were recovered from the *Bt*-sprayed plots, and these were *C. flavipes* (2.6%) and *Pediobius furvus* (1.8%), and two parasitoid species *C. sesamiae* (0.05%) and *Cotesia ruficrus* (Haliday) (0.23%), were recovered from the CI treated maize.

Results of this study show that *Bt*- spray was more effective than the conventional insecticides (CI) in controlling stem borer damage in maize. The smaller range of parasitoids that were recovered from the *Bt*- and the CI sprayed maize, compared to the untreated one, suggests that both these treatments have some level of effects on some parasitoids of stem borers. Although a similar number of parasitoid species was recovered from the *Bt*- sprayed and CI treated maize crops, the type of species was different, thus indicating their differential effect on the parasitoids.

Another study was made at CIMMYT's headquarters in Mexico where a biosafety green house and laboratory are available to conduct transgenic trials. The experiment was designed to develop a methodology to test the impact of Bt maize on biological control agents and to identify a potential synergism between a Bt maize and a wasp which attacks a species of armyworm (*Spodoptera frugiperda*) which is similar to the armyworm species found in Kenya (*Spodoptera exigua*). Two experimental protocols were used: 1) no-choice in which the armyworm and the wasp (*Campoletis sonorensis*) which attacks its parasitoids were placed on maize leaves only with Bt (*Cry IAb* toxin) or without Bt; and, 2) a free choice experiment in which the wasps were placed inside a netting which contained both Bt and non-Bt maize infested with armyworm.

The rate of parasitism does not change between Bt and non-Bt maize, with both types of maize resulting in a peak rate of parasitism around 45% attained 10 days after armyworm placement on the plants (Figure 1). The second observation is the higher rate of parasitism on Bt maize following the peak, with 30% parasitism being observed on day 12 versus only 20% for the non-Bt maize. The reason for this difference is the reduced growth rate of the armyworm when feeding on the Bt maize. The average weight of the armyworm on day 12 was 9.7 mg for those collected on Bt maize versus 16.6 mg for those on non-Bt maize. The significance of this observation is the fact that the wasp has a long period of time to attack the armyworm feeding on the Bt maize. The significance of this observation is the fact that the wasp has a long period of time to attack the armyworm feeding on the Bt maize as once the armyworm reaches the third larval instar it is too large to be successfully attacked by the parasitoid. In this regard, the Bt maize is enhancing the efficiency of the wasp in controlling the armyworm even though the Bt maize is not directly controlling the armyworm pest. Another possible advantage to this system is the fact that with a reduced growth rate, the rate of cannibalism, which is known to occur in armyworm species, is reduced on the Bt maize. This would mean that armyworm which have been attacked and have the wasp larvae developing within them could likely have a great probability of escaping consumption by neighboring armyworm and therefore facilitate higher parasitoid populations. This hypothesis has not yet been tested but will be the subject of future testing.

This study has now established a protocol for testing the interaction between parasitic wasps and transgenic maize to quantify their impact on the control of secondary pests of maize, such as the armyworm. Once a biosafety greenhouse is in place in NARL, these types of studies will be continued in order to quantify the environmental impact of Bt maize on biological controls and other non-target organisms found in Kenya.

Product Dissemination – Development of appropriate insect resistance management strategies for resource-poor farmers in Kenya:

To counter the buildup of resistance by the borers to Bt maize, we are developing varieties that carry multiple forms of resistance—for example multiple Bt genes and combinations of Bt genes as well as conventional resistance. So a borer population would have to develop multiple resistances rather than a single resistance to the Bt. In addition, management strategies are being developed, with the help of farmers, that maintain populations of non-resistant borers that will breed with potentially resistant borers and limit the buildup of resistant populations. Any host of the borers can be used for this purpose and taken collectively they are known as "refugia".

Gould (1998) has discussed at length some of the theoretical aspects of genetically engineered crops for durable resistance, Gould (1986) has also used simulation models to evaluate different resistance management strategies. Generally, mechanisms of resistance management strategies are based on three principles; diversification of mortality sources so that a selection pressure is divided between multiple mortality factors (Georghiou, 1972), reduction of selection pressure from each mortality factor/mechanism to the target pest (Wharlon and Norris, 1996) and maintenance of susceptible pest individuals by providing refugia or promoting immigration of susceptible pests (Wharlon and Norris, 1996).

Research is needed to determine how much refugia from Bt must be provided in space and in time to slow resistance development substantially. The refugia area depends on the crop and the selected type of refugia treatment. Most cereal stem borers of maize and sorghum are polyphagous and have several graminaceous and other wild hosts in addition to cultivated crops. Wild host plants of stem borers have been documented by various workers (Ingram 1958, Bowden 1976, Seshu Reddy 1983, Khan *et al.,* 1997). The most important alternative hosts of the major stem borers (*B. fusca, C. partellus, S. calamistis and E. saccharina*) are reported to be cultivated sorghum, *Sorghum versicolour, sorghum arundinaceum,* Napier grass (*Pennisetum purpureum*) and *Hyperrhenia rufa* (Khan *et al.*, 1997).

Although stem borers oviposit heavily on some grasses only a few grasses are favourable for them to complete their life cycles (Huttler, 1996). It is therefore very important to select alternative hosts with economic value, e.g. high yielding livestock feeds or food crops which fit into the farming systems where the Bt maize will be planted. Studies on development rates of different stem borers have been done by Khan *et al.*, (1997) on maize and a few Napier grasses mainly for *Chilo partellus*. However, there is need to study development and survival rates of the common stem borer species in various agro-ecological zones on various grasses of economic value. This information is necessary to be able to synchronize the mating between susceptible insects from the refugia (forages) with the resistant insects emerging from Bt. maize. It is also important to recommend to farmers the cutting regimes for Napier grass and other forages based on the development time, to avoid harvesting Napier before the pests complete their life cycle.

Studies were, therefore, initiated within the IRMA Project to develop insect resistant management (IRM) strategies for Kenyan ecosystems based on existing cropping systems. To be accepted by farmers, IRM strategies must conform to existing cropping systems, and the refugia crops must be economically viable and socially acceptable to those making the management decisions at the farm level. Studies focussed towards verifying these tenets were also initiated. After evaluating 30 different alternate hosts for stem borers, preliminary results show Columbus and Sudan grasses as the most effective refugia for *C. partellus* and *B. fusca.* Sorghum was the best host for *Chilo* and *Busseola*, given the large number of exit holes per stem and numerous tillers. Napier grasses attracted oviposition, but were not good hosts for larval development.

Gene flow, the movement of genes between plants of the same species:

This is particularly found in cross pollinated crops like maize. Research is underway to estimate the distance that pollen travels and to assess the methods farmers use to select seeds with respect to the relative location in the field.

Most farmers in Kenya recycle seed for planting the following season. This has several implications for IRM. Unlike developed countries where farmers sign licensing contracts at the time of seed purchase, farmers in developing countries are not likely to report resistance breakdown. Therefore, techniques must be developed that will enable the early detection of resistance development so steps can be taken to replace the technology in a timely manner to avoid resistance breakdown. Screening technologies should be inexpensive and sensitive enough to detect shifts in the insect populations in a timely manner. A sampling protocol must also be developed to ensure that representative samples are taken from the major maize growing regions, especially those that have a high adoption rate of Bt technology. Agronomic studies will commence when insect resistant maize varieties are available. Seed production strategies will be developed when insect resistant maize varieties are available.

Impact Assessment – Assess the impact of insect resistant maize varieties in Kenyan agricultural systems:

IRMA's impact assessment group of social scientists have focussed on assessing various aspects of insect losses, suitability and demand of the new insect management technologies, farmers' perceptions of crop losses and control options, and assuring that the technology fits within Kenya's institutional framework. Through continuous dialogue with different stakeholders such as environmental groups, local research institutes, seed companies, and above all the farmers, IRMA has gained a clearer understanding of social, environmental and economic impacts of insect resistant maize in Kenya.

Participatory Rural Appraisals (PRAs) organized in the five maize growing ecological zones of Kenya have identified farmers' preferences for maize varieties and the constraints they face. Group interviews and discussions with more than 900 farmers were conducted. Over all the zones, most farmers plant local varieties in the low-potential areas while improved varieties dominate the high-potential areas. The most important selection criteria are early maturity and yield, followed by drought tolerance, then tolerance to field and storage pests. The major constraints to maize production were availability of cash, lack of technical know-how, and availability of good quality seed. The major pest problems, according to farmers, are stem borers and weevils. Farmers show a keen interest in new insect resistant varieties if they fit their selection criteria, even if they are moderately more expensive. However, since seed supply and quality are problems, the quality of seed needs to be guaranteed.

Results of our maize sector study show that most restrictions on maize marketing have been lifted, and that markets for fertilizer and pesticides are fairly free. Poor infrastructure, market information, and access to rural credit markets remain problematic.

Average crop loss at the national level due to stem borers is 15%, with a value of US$ 91 million, according to a calculation based on farmers' estimates. IRMA also conducted overall crop loss assessment trials in farmers' fields. The measured yield difference between plots provides an estimate of the loss due to stem borers and is estimated at 14% a value of US$ 60 million in 2000. These trials were repeated in 2001, and preliminary results show losses between 6.5% in the highlands and 10.5% at the coast. Crop loss assessment will be a continuous exercise in the IRMA project to ascertain the losses experienced by farmers.

Stem borers are the most widely distributed and most damaging pests to maize worldwide. In Africa, there are several economically important stem borer species. The most important borers are the spotted stem borer (*Chilo partellus*) and the African stem borer (*Busseola fusca* Fuller). *Chilo partellus* is found mainly in East Kenya, and is the most destructive pest of maize in warm, low-altitude regions. *Busseola fusca is* mainly in West Kenya, is native to Africa and is the major borer pest in the highlands. A very important aspect of the IRMA project is that the work carried out by KARI and CIMMYT will be used to help other African countries in the region combat maize stem borers. The IRMA project will be working in all maize production regions to develop maize varieties that both offer resistance to the most important stem borers in a given region and also produce good yields under local growing conditions. We plan to make the experiences and lessons learned -- and some of the maize germplasm that we develop in this project -- available to those of our neighbours that want to use this technology themselves.

Technology transfer, awareness creation, and communications:

In any undertaking involving new technology and technology transfer, capacity building in local institutions is critical to success and sustainability. Training of KARI scientists was done through visits to Mexico and on-site in Kenya on genetic engineering, management of biosafety and entomology laboratories and on how to conduct insect bioassays. Others were exposed to biosafety regulatory systems (development, dissemination and enforcement with the Mexican example). Other training was on impact assessment and general methods in breeding and entomology. Infrastructure support and development were realized through the planning of biosafety laboratories at the KARI Biotechnology Center, development of a biosafety level 2 laboratory, provision of logistical facilities like computers, vehicles, laboratory equipment, and support of insectaries and entomology laboratories. In a project where new technology is being developed and disseminated, communication is important for education and creating public awareness. Considerable effort has been given to creating dialogue and raising public awareness about biotechnology in general and Bt gene-based stem borer resistance. Stakeholders meetings, establishing positive media relations to achieve objective coverage, creation of print and electronic materials, working closely with local press, and participation and documentation of relevant seminars and conferences are some of the ways used to enhance communication.

CONCLUDING REMARKS

The approach of the IRMA project to the issue of risk is to conduct research to address issues within our capability, draw from experiences elsewhere, and collaborate with partners with necessary expertise on issues not easily addressed by the project staff. African scientists are generally positive towards the use of GMOs (Ndiritu and Wafula, 1998; Wambugu, 2000). Opposition to GMOs stems mainly from suspicions that not all stakeholders will benefit. Farmers particularly in developing countries, stand to gain from increased production coupled with reduced costs of production. The consumers may not feel the benefits of increased production especially in developed countries where food is plentiful and costs are low. Recent reports indicate that food made from genetically modified crops do not pose greater risks to human health than those made from non-genetically modified crops. The World Health Organization, the UN's Food and Agriculture Organization, the United States Department of Agriculture and others have declared that the Bt maize foods now on the market are safe (NRC, 2000). To avoid any undesirable effects of antibiotic resistance in Bt maize foods and feed, CIMMYT is developing genetically engineered germplasm without antibiotic resistance genes.

The IRMA project has considered risks to the environment. For Bt products, such risks include: 1) impacts of the Bt maize on non-target organisms, 2) potential development of resistance of stem borers to the transgenic Bt maize, and 3) potential of gene-flow from the transgenic maize to other cultivated and/or wild plant species (Serratos *et al*., 1997). These concerns have been addressed through activities reported here. Risks of Bt maize monoculture in Kenya are relatively low as adoption levels for any technology in maize production is usually lower than expected.

The concern about gene flow relates to gene transfer to other plant species, with possibilities of creating super weeds, "contaminating" landraces with transgenic maize, or by reducing diversity in the environment through greater competitiveness of Bt maize. Studies are under way to address the issues on gene flow. However, gene flow into other species is of less consequence in Kenya, as maize is not native to Africa and there are therefore no wild relatives that would readily cross to maize.

Development of an insect resistance management strategy will address the buildup of resistance by the borers to Bt maize. Management strategies such as the use of refugia help to forestall the development of resistance in stem borers. Varieties that carry multiple forms of resistance such as combinations of Bt genes (pyramiding resistance) and conventional resistance will be developed. Thus, a borer population would have to develop multiple resistances rather than a single resistance to the Bt toxin. This should greatly slow the build-up of resistance to the Bt toxin. Yet another strategy to be pursued is the use of two-toxin Bt maize, both at high doses. If stem borers that are able to survive on a plant with one high-dose toxin are rare, then those that will survive on a plant with two high-dose toxins will be even rarer. Research done at CIMMYT in Mexico has shown that larvae of *Diatraea grandiosella* and *D. saccharalis* will not survive beyond 8 days on Bt maize after 18 cycles of selection on Bt maize (Bergvinson, 1999). Studies are underway on dispersal behaviour of stem borer and the feasibility of natural habitats as refugia.

To address the concerns surrounding Bt maize, we have taken various approaches: 1) gathering information from various sources in literature, 2) informing stakeholders through meetings, exhibitions, published literature and newsletters, 3) carrying out field research where significant gaps in information exist, and 4) strictly adhering to national regulations at all stages of project development.

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