SESSION 10B

THE POTENTIAL ROLE OF TRANSGENIC CROPS IN SUSTAINABLE AND DURABLE PEST AND DISEASE MANAGEMENT

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Transgenic crops: can European consumers benefit from eating them and will they want to?

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ABSTRACT

The impact of possible future commercial introduction of food products produced from transgenic crops on European Consumers is analysed under two scenarios. The '*benefit from eating*' scenario assumes consumers are indifferent to the choice implicit between the consumption of products derived partially or wholly from transgenic crops and other non-transgenic products. Benefits under this scenario arise from changes in the full budget constraint of the household (e.g. lower food prices, higher nutritional value). The '*will they want to*' scenario assumes that consumers' preferences differ between goods derived partially or wholly from transgenic crops and other non-transgenic products. Consumer surveys about preferences towards those goods are reviewed and compared.

INTRODUCTION

Agrobiotechnology is providing political challenges to agriculture in many countries. Never before has a new technology in the field of agriculture been so emotionally debated among different stakeholders. Scientists from developing countries fear that they will be bypassed by the new technology. At the same time groups of consumers, politicians and non-government organizations (NGOs), both in developed and developing countries, oppose the introduction of transgenic crops, which they see as a threat to biodiversity, human health and the economy of rural communities, ultimately endangering sustainable development. Radical groups go as far as destroying research plots and laboratory equipment. Especially in Western Europe, many people have lost their confidence in modern science because of the BSE scandal, HIV-tainted blood and other such incidents. Consumers are further disconcerted by the disagreement among scientists about the environmental and human health impact of transgenic crops. While some highlight the potential risks, others argue that they are negligible.

However, much of the discussion on the risks and benefits of agrobiotechnology is based on ideologies and beliefs. Scientific evidence is scarce, and economic analyses are at a very early stage of providing guidance to policy makers and other stakeholders.

In this paper the economics of agrobiotechnology will be discussed, with special emphasis on the impact on consumers. The impact on consumers cannot be disentangled from the economic characteristics of transgenic crops or agrobiotechnology in general. Therefore, firstly, important economic characteristics of agrobiotechnology are presented. This is followed by a discussion of the impact at research and development, agriculture sector and

consumer level and the distribution of benefits and costs. Lastly, the main conclusions and an outlook to future trends and research priorities are presented.

IMPORTANT ECONOMIC ASPECTS OF AGROBIOTECHNOLOGY

From an economic point of view, the two most important aspects of agrobiotechnology that need to be considered relate to issues of efficiency and equity. Efficiency looks at the impact of agrobiotechnology on resource allocation and productivity within the economy, while the question of equity attempts to analyse the benefits and costs of these new technologies and how these will be distributed among different stakeholders. The three main stakeholder groups who are affected by or have an interest in agrobiotechnology are shown in (Figure1) and include:

(1) the providers of the technology, namely universities, other public research institutions and private companies
(2) the farmers as the main users of the technology
(3) the consumers as those who are confronted with the final products

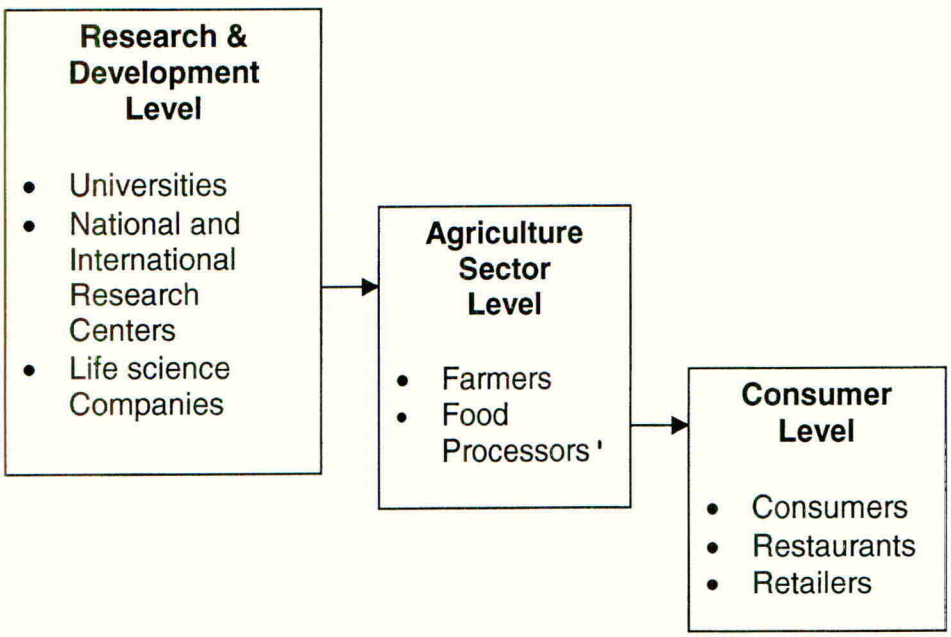

Figure 1. The main stakeholder groups in the agrobiotechnology chain

The questions regarding efficiency and equity can therefore be discussed at the level of research and development, at the production (= agricultural sector) level, and at the consumer level. In addition, national governments and international organizations as the regulatory bodies that have the power to influence the distribution of costs and benefits of the new technology also have to be considered. Furthermore, since agrobiotechnology will not only have an impact on western agriculture and society, but also on those of developing countries, who expect large benefits from its application, the conditions under which those benefits will materialize for the benefit of developing countries are of particular interest.

RESEARCH AND DEVELOPMENT LEVEL

Public research institutions in developed countries have discovered the basic foundations of agrobiotechnology. However, the introduction of patents and other Intellectual Property Rights (IPRs) for biotechnology inventions provided an incentive for private companies to invest in the technology, so that now private investments in agrobiotechnology research are many times greater than those of the public sector. A patent puts its owner in the position of a temporary monopolist for the supply of a specific product. For as long as the patent is valid, the owner can exploit monopolistic profits. This situation can be justified by the high initial investments needed to generate an invention. Without intellectual property protection, the private sector would have no incentive to invest in research and less technical change would be generated.

The nature of agrobiotechnology, which relies on seeds as the carriers of the invention, has resulted in several mergers and acquisition (M&As) between biotechnology and seed companies. Biotech companies, which were able to incorporate new traits into existing germplasm, did not have the seed distribution system necessary to capture the gains from their new developments. In order to bring their products to the market, biotech companies could either enter into contracts with seed companies, or they could actively engage in this part of the development process through vertical integration, i.e. by buying into the seed distribution system through M&As with seed companies. The latter option became dominant, as specific transaction costs could be considerably reduced through M&As. However, this situation of concentration has given rise to concerns among many critics of agrobiotechnology as they see the market power of multi-national biotech-cum-seed companies as becoming overly strong.

The growing involvement of private companies in agrobiotechnology research has also given rise to many new forms of public-private partnerships. These partnerships have changed the research sector in the US, especially with respect to the land-grant universities. The role of public research is put into question as the share of privately financed research projects at public research institutions increases. On the one hand, public research institutions need partnerships with private companies to access the protected germplasm, molecular tools and processes owned by these companies, but also they need to commercialise their own research findings for public benefit. On the other hand, the independence and character of public research and the character of public research public good is threatened by too much private sector involvement. Most notable in this respect is the contract between the University of California Berkeley's College of Natural Resources and the life-science company Novartis, in which Novartis made an initial commitment of US$25 Million to fund research and obtained the right to negotiate licenses on the research

results. The structure of such emerging public/private partnerships is also important for the development of agrobiotechnology for developing countries where private investment in agricultural research remains negligible. Many life-science companies hold property rights on genetic material of world food crops like rice or corn. This limits the research possibilities of public institutions, including the international agriculture research centres. Partnerships between the private sector and national and international research centres have been discussed to improve the research potential of the centres.

AGRICULTURE SECTOR LEVEL

There are three important aspects that have to be considered when analysing investments in agrobiotechnology at the farm level. First, investments in agrobiotechnology are done under temporal uncertainty, second they are to a certain degree irreversible, and third they can be postponed into the future. While the first aspect concerns mostly the farmers' decision to use a transgenic variety, the latter two aspects become important at the level of society in the decision on whether or not to release a transgenic variety for public use.

Temporal uncertainty exists since future prices, yields, and costs of the new products are unknown. The price of genetically engineered crops may increase or decrease compared to "conventional" varieties for a number of reasons including consumer reactions or government regulations. For example, the relative price of transgenic varieties may decrease if consumers are willing to pay a premium for so called GMO-free products. On the other hand, the relative price may also increase if a growing number of consumers believe that GMO products have a higher value than non-GMO products, for example because of higher nutritional value. On the production side, the relative variable costs may increase or decrease depending on prices for the different inputs needed as well as differences in production technology. For example, the culture of herbicide-tolerant plants may reduce the number of herbicide applications and hence reduce the variable costs for hydrocarbons to run farm machinery. Furthermore, the relative changes in yield are unknown. All three, product prices, variable costs, and yields, contribute to the farmers' uncertainty about the relative changes in future gross margins. In addition, regulations regarding the development, release and use of agrobiotechnology products may change over time. As additional information on the environmental impact of transgenic crops becomes available, regulating agencies will start to implement guidelines for their use, which may add additional costs to the producer, processor or developer.

Irreversibility exists as a release of genetically modified organisms may have a negative impact on the environment. There are numerous risks related to the widespread use of transgenic crops. For example, gene flow in plants may enable domesticated plants to become pernicious weeds, or it could enhance the fitness of wild plants, which might turn out to be serious weeds, thus shifting the ecological balance in a natural plant community. New viruses could develop from transgenic crops transformed with virus genes. Plant-produced insecticides might have harmful effects on unintended targets. While some of these scenarios are highly unlikely, little is known about the overall impact that transgenic crops can have on biodiversity, ecosystem balance and the environment. Once released into the environment, the new genetic information cannot be readily removed.

In the United States, transgenic crops have been adopted rapidly (James, 2001). Studies confirm that on average the gross margin per unit area from transgenic crops is about as high, and is sometimes higher than the gross margin from non-transgenic crops. However, there is a regional difference in the distribution of benefits, which can be explained by regional factors such as the infestation level with pests and weeds and the climatic conditions. The empirical studies also indicate that the amount of pesticides used may decrease for transgenic crops but only in specific regions and specific years, depending on the same factors as mentioned above. In some regions, pesticide use has actually increased.

The rapid adoption of transgenic crops among farmers in the USA has been explained by the greater benefits that farmers gain from planting transgenic crops. Variable production costs are reduced because of reduced pest management and labour costs. Gross revenues are increased because of an increase in yield from improved plant spacing. Additional benefits arise from improved risk management and insurance against pests and a reduction in equipment costs in zero-tillage production systems.

The decrease in pesticide use not only reduces the expenses for farmers but also reduces the pressure on the build-up of pesticide resistance. Additionally, the reduced application of pesticides has several positive impacts on the environment and human health although these may be difficult to quantify in financial terms. The reduced pressure on the build-up of pest resistance and some of the other external costs of pesticide application are irreversible. If the introduced transgenic crops result in less pesticide application, the introduction provides additional benefits. Hence, the release of transgenic crops produces not only irreversible costs but also irreversible benefits. That is, there is a trade-off from the economic point of view from releasing transgenic crops between the increase in pest susceptibility, because of a decrease in pesticide use, and the increase in resistance to pesticides and antibiotics, because of the planting of transgenic crops. Uncertainty, irreversibility and the possibility to delay the release of transgenic crops have an impact on the optimal timing of release as over time additional information arrive (Wesseler, 2002).

CONSUMER LEVEL

Uncertainties on the impact of agrobiotechnology abound at the consumer level as well. The first generation of transgenic crops, which focused on herbicide tolerance and pest resistance as the dominant traits, does not provide any significant direct benefits for the consumer. Food prices will not decrease as long as the share of primary agricultural products in the total costs of processed consumer goods is very low. Currently, the share of wheat in the costs for bread is below 10 percent, the other costs are accounted for by processing, transport, and packaging. Therefore, it is understandable that consumers are reluctant to buy products containing transgenic crops, more so as they do not have any direct positive impact on health but, on the contrary, are perceived as being risky to consume (e.g. Moon & Balasubramanian, 2001). An experimental study confirmed the translation of perceived risk in a lower but positive willingness-to-pay for otherwise similar products including ingredients from GMOs indicated by a label (Noussair *et al.*, 2002). Whether or not this observed behaviour will continue in the long run is not certain. It is more likely that consumers will turn their attention towards comparing GMO and non-GMO products and become indifferent as the observation about other food issues like the BSE ('Mad Cow

Disease') in Europe or administration of recombinant bovine somatotropin (rBST) to milking cows in the United States indicates.

Consumers are affected in another way as well. Broadly speaking, consumers can be divided in two groups. One that would not buy any food made from transgenic crops and one that is indifferent or has a positive willingness to pay. Both groups are affected differently under different regulatory systems. For simplification the following will be summarized under the term GMO-food, (1) food made from transgenic crops, (2) food including ingredients derived from transgenic crops like highly refined sugar and oils and meat and dairy products from livestock fed on transgenic crops. If GMO food is not labelled, those who prefer not to eat GMO food will not be able to identify non-GMO food if the market does *not respond* and would have to eat them anyhow, even if they do not want to. But under this scenario the market *would respond* and provide non-GMO food as it already does by offering organic food.

If GMO-food will be labelled, those who prefer non-GMO food will be able to identify their choices. As labelling is not cost-free, part of the costs will be transferred to the consumers. Those who are indifferent about GMO-food will loose, as they have to pay a higher price. The problem is further complicated by the observation that the willingness to pay for non-GMO food differs among consumers. The increase in the consumer price of labelled GMO food may be higher than the willingness to pay of some of the consumers and they will also loose. In the case of voluntary labelling in the sense "GMO free food" the price of non-GMO food rises and those who prefer them have to pay a part of the price increase and will be worse off. The correct labelling procedure is difficult to answer from an economic point of view, but so far the most cost effective procedure is to allow for voluntary labelling. This argument is supported by the observation that the market for food that includes positive environmental attributes, like organic food, is very small.

The approach in the European Union is to label food with more than 0.5% of material from transgenic crops. The European Parliament has recently rejected compulsory labelling of non-GMO food. Given the previous discussion, the approach by the EU will reduce on the one hand the freedom of choice of those that are indifferent towards GMO food, as they have to pay part of the labelling costs. On the other hand, this group may gain, if food prices decrease due to the use of transgenic crops, but this is, as mentioned earlier, very unlikely. Those who prefer non-GMO can identify GMO-food but not non-GMO food. They will loose compared to the situation without GMO-food, as the market for non-GMO food decreases and hence prices increase. With mandatory labelling of first generation transgenic crops there will be no direct positive net-benefits for consumers compared to a situation without GMO-food. Consumers will be only net-beneficiaries, if the indirect net-benefits of transgenic crops are positive and outweigh the direct negative aspects. In a dynamic setting the consumers may also gain indirectly from positive net-benefits at the up-stream agriculture and research and development level.

The second generation of transgenic crops is expected to provide more direct benefits to consumers, for example through improved nutritional contents of the crops or as functional food, e.g. plant protein transformed in a meat-like product. It still has to be shown if consumers in European countries will accept this as a benefit, since they already have alternatives for a balanced nutrition. Most of the benefits from the second generation of

transgenic crops at the consumer level are expected to be realized in developing countries, where problems of nutrition deficiencies can be addressed through products such as Vitamin A- enhanced transgenic rice.

DISTRIBUTION OF BENEFITS AND COSTS

The potential net-benefits agrobiotechnology promises to society should not be discussed without having a closer look at the distribution of those net-benefits. Again, if the economy is divided into the three groups comprising researchers, farmers, and consumers, which group will be the main beneficiary? Empirical studies on the distribution of net-benefits from Bt-cotton showed that farmers were the group receiving the highest share from the overall net-benefits, followed by the agrobiotechnology industry and lastly the consumers (Falck-Zepeda *et al.*, 2000). As Bt- cotton belongs to the first generation of agrobiotechnology, it can be expected that the net-benefits at the consumer level will increase with the introduction of second-generation agrobiotechnology products. The distribution of benefits also has an international dimension. As most of agrobiotechnology is currently applied in Northern America, producers and consumers in this region will be the main beneficiaries. However, other regions of the world will benefit as well, depending on the structure of their current agricultural production. As the prices for transgenic agricultural commodities will most likely decrease, those countries that are net-importers of those crops will benefit, specifically the EU and Japan and other developed countries. Among the developing countries, China will most likely be the main beneficiary. Developing countries who are net exporters of agricultural products are expected to be the loser. Also, the producer surplus of net-food producing farmers in developing countries is expected to decrease as well, while the surplus of consumers in urban areas is expected to increase. The total net benefits at the national level of developing countries will hence depend on the urban-rural population ratio and the level of net-food production.

CONCLUSIONS AND OUTLOOK

The development and application of agrobiotechnology has important implications for the organization of research, the economics of agricultural production, and consumer welfare. The role of public-private partnerships in research will become more important in the future and will challenge the independence of public research. The concentration among the life-science companies through mergers and acquisition has to be observed closely to avoid excessive use of monopolistic power. This will be of special concern for regulators, as they have to weigh the gains through patents against the welfare losses caused by use of restricted monopolies.

Until now, one of the major problems in the economics of agrobiotechnology is the assessment of benefits and costs at the farm level. The nature of the problem demands analytical methods that have only been developed recently. The incorporation of irreversibility and uncertainty allows researchers to recognize the risk associated with the release of transgenic crops into the environment at the theoretical level. Further research in this direction will improve the quality of the assessment.

It is still uncertain if consumers will accept food products made from transgenic crops. They show on average a positive willingness-to-pay for non-GMO food. Experiences in the US with rBST-milk suggest that negative labelling like "milk from cows not treated with rBST/rBGH" may be a solution and create niche markets (Runge & Jackson, 2000). The current approach of the European Union for mandatory labelling of GMO-food will result in a loss of direct consumer net-benefits. If indirect net-benefits in a dynamic setting are included consumers may gain.

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Transgenic crops and integrated pest management

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ABSTRACT

Integrated pest management (IPM) emerged several decades ago in response to the mounting environmental problems with the injudicious use of pesticides. The concept is to combine the use of different, necessity-driven control strategies, including the use of cost-free natural control processes, thereby reducing dependency on a single solution, i.e. pesticides. IPM re-introduces ecology into industrialized agricultural production systems. Chemical control measures should only be used when all other non-chemical measures fail. The introduction of transgenic crops into the agricultural systems of some countries pose a number of critical challenges that will be discussed from the point of view of if, and how, current and future transgenic plants comply with the IPM concept. Two components will be instrumental for the successful incorporation of novel, transgenic crop plants into IPM systems, a) appropriate pre-release testing methodologies for non-target effects that provide meaningful data for ecological risk assessment and b) post-release monitoring and scouting programs that ensure continued oversight of the predicted (beneficial or adverse) impacts – or the lack thereof. An illustrative case example is discussed. It is concluded that if transgenic crops for crop protection are to become an integral component of IPM systems, comprehensive plans for their incorporation have to be developed and communicated to the farmers prior to their large-scale commercial release.

INTRODUCTION

Integrated pest management (IPM) was introduced as an answer to the development of pesticide resistance in pests and the increasing environmental problems associated with pesticides following their large-scale use since the 1940s. According to Levins (1986), IPM represents a '... softening of a stance of hostile confrontation with all of living nature except the crop, and a groping toward a strategy of détente and coexistence with most species'. The concept of IPM is based upon a variety of methods integrated in a way that reduces dependence on a single solution, such as a pesticide. These methods include evaluating pest management according to economic threshold levels, using cultural control methods (e.g. crop rotation, mulching), host plant resistance, mechanical control methods (e.g. ploughing of infested plant residues), biological control (e.g. use of natural enemies), microbial control (e.g. Bt-based insecticides) and, lastly, the judicious use of synthetic insecticides only if none of the above methods work acceptably. Over the past three decades, much time, work and resources have been devoted to developing and implementing IPM systems worldwide. *Agenda 21*, the blueprint for the environment in the 21st century agreed by governments at the 1992 Rio Earth Summit, states that "integrated pest management, which combines biological control, host plant resistance and appropriate farming practices and minimizes the

use of pesticides, is the best option for the future, as it guarantees yields, reduces costs, is environmentally friendly and contributes to the sustainability of agriculture".

Several years ago, another technology with potentially wide-ranging ecological implications – the use of transgenic crops - entered the agricultural production systems of some countries and is poised to enter European agriculture. So far, 99% of all transgenic plants worldwide are grown in 4 countries only, USA, Argentina, Canada, and China (in descending order) (James 2001). IPM is practised widely in many European countries, and one of the great challenges of the coming decades is to explore how these novel transgenic plants could be incorporated as safe and effective components of sustainable IPM systems. Existing and future transgenic plants need to be evaluated carefully to determine if and how they fit into the IPM concept. For example, is constitutive expression of high concentrations of an insecticidal compound (i.e. season-long high persistence of an insecticidal toxin in almost all plant parts) compatible with the IPM philosophy of controlling insects at or below an economic threshold and otherwise allowing for coexistence? Similar questions have been raised for herbicide-tolerant plants, the employment of which is coupled to the use of a particular, complementary herbicide. In the case of the stacking of herbicide-tolerance and Bt toxin production, a farmer who needs herbicide tolerance only (which may be not available) may end up planting a crop with the Bt-trait, even if densities of the target pests on the farm do not warrant control with the Bt trait. There is evidence that for up to 20% of the cotton farmers in the southeast of the US, this may be the case with stacked herbicide tolerant Bt-cotton (Anonymous, 2002). Obviously, economic threshold levels are irrelevant in such production systems, as is necessity-driven pest or weed management.

Two components seem to be instrumental for the successful incorporation of novel, transgenic crop plants into IPM systems:

a) appropriate pre-release testing methodologies for non-target effects that provide meaningful data for ecological risk assessment and
b) post-release monitoring and scouting programs that ensure continued oversight and the integrated use of these plants.

PRE-RELEASE TESTING METHODOLOGIES FOR NON-TARGET EFFECTS

Non-target effects include any unintended side effects of transgenic plants on organisms other than the target species. These non-target species may include detritus-feeding organisms, pollinators, and other herbivores, as well as higher trophic level organisms such as the insect natural enemies of both the non-target herbivores and the original target species. Undesired non-target effects can interfere with the processes that naturally regulate herbivores and higher trophic level organisms (e.g. predators and parasites) and can have implications for biocontrol and IPM programs (Hilbeck, 2002).

A comprehensive characterization of the transgenic plant and a sound understanding of the input and fate of the released plant material and the expressed novel protein are pre-requisites for developing adequate non-target testing methodologies. The potentially exposed or affected non-target organisms can be identified using a theoretical causal chain of exposure and impact based on the knowledge of the transgenic plant, and a working knowledge of the biota associated with the crop and their functions in the target agroecosystem. The testing organism should be a dominant or important species in the target ecosystem. Wherever

possible both laboratory and field tests should be carried out to test similar hypotheses. This is because of the difficulties inherent in extrapolating laboratory data to the field.

Focus on non-target natural enemies

When non-target herbivores ingest a novel insecticidal compound, such as a *Bacillus thuringensis var. kurstaki* (Berliner) toxin, they can affect their natural enemies in various ways (Figure 1):

1) the insecticidal compound or any metabolite of it may affect the natural enemy directly;
2) the insecticidal compound exerts an interaction effect in concert with other secondary or primary compound(s) of the plant;
3) the insecticidal compound affects the nutritional quality of the prey or host herbivore and thus affects the natural enemy indirectly, or
4) Any combination or all of the above may affect the natural enemy.

It will be very difficult to distinguish between these different levels of impact. Ultimately, what matters is whether or not the fitness of a species and thus its continued existence will be affected. The most encompassing evaluation of potential effects on natural enemies can be accomplished if the experiments involve the whole transgenic plant and herbivores that fed on the transgenic plant. The case example in the next section below will illustrate the different hazard outcomes that are possible with different exposure routes.

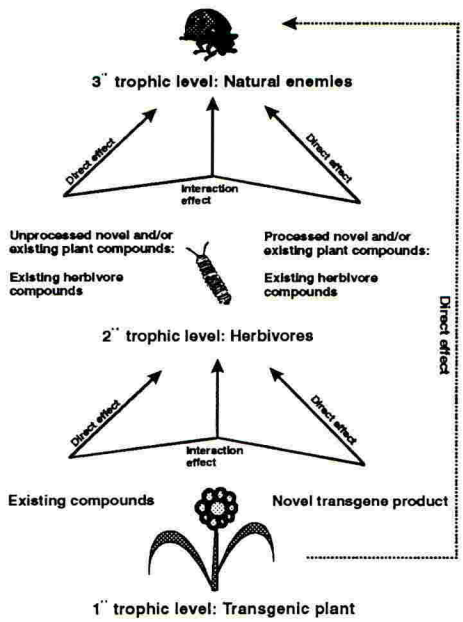

Figure 1. Potential tri-trophic interaction effects on natural enemies.

Altered non-target herbivore population dynamics induced by the impact of the insecticide expressing transgenic plant can also affect natural enemies. This will be most important for those natural enemy species that follow their prey population dynamics in a density-

dependent fashion. These are often specialized natural enemies, such as parasitoid species that only feed on one host species. If this non-target host species declines in density or is driven to local extinction because of the impact of the expressed insecticidal toxin, their specialized parasitoids or predators will also decline or go extinct. Evidence for this from laboratory and field trials has been published (Schuler *et al.*, 1999, Riddick *et al.*, 1998). Some argue that the local extinction of a specialist natural enemy may be an acceptable sacrifice if the target pest is also eliminated. However, this is a risky ecological speculation because we typically know little to nothing about the ecology, function and activities of these specialized natural enemies outside the crop field. The lack of certain natural enemy species may become evident unexpectedly and result in adverse effects in an entirely different ecosystem context. On the other hand, polyphagous natural enemies, such as many predator species, can simply switch to other prey species that happen to be available in the agro ecosystem, and it can therefore be speculated that they are less likely to go extinct through lack of prey, though for example they could decline or go extinct due to the adverse affect of the novel insecticidal compound passed on to them through their prey (see below 'Risk Assessment').

CASE EXAMPLE: NON-TARGET EFFECTS OF TRANSGENIC Bt-PLANTS AND MICROBIAL Bt-PREPARATIONS ON *CHRYSOPERLA CARNEA*

The effects of transgenic *Bacillus thuringensis* (Bt) - expressing corn and microbially produced Bt-proteins on an important, very polyphagous natural enemy species, the green lacewing *Chrysoperla carnea* (Stephens), were studied in bi- and tri-trophic experiments. Three series of no-choice experiments were carried out using different Bt-delivery systems - transgenic Bt-corn and Bt-incorporated diets. This allowed comparison of the effects of consumption of herbivorous prey eating a Bt-containing diet and the direct effects of a Bt-toxin on *C. carnea* larvae. Additionally, three series of choice experiments were carried out where *C. carnea* larvae could choose between aphids (*Rhopalosiphum padi)* (Homoptera: Aphidae) and lepidopteran larvae (*Spodoptera littoralis)* (Lepidoptera: Noctuidae) raised either on Bt- expressing corn or the corresponding isogenic untransformed Bt-free corn hybrid.

No-choice trials

The results of all three series of no-choice experiments consistently demonstrated the susceptibility of immature *C. carnea* to Bt proteins (Cry1Ab toxin and protoxin, Cry2A protoxin), whether provided *via* prey or directly (Hilbeck *et al.*, 1998a,b, Hilbeck *et al.*, 1999). The degree of mortality varied depending on the Bt-delivery system, and an increase in toxicity of the Bt-protein through the food chain was observed (Table 1). Prey-mediated mortality of immature *C. carnea* was highest when the prey food source was transgenic Bt-corn (59 - 66%) relative to the concentration of the Bt-toxin Cry1Ab, which was the lowest in plants (<5 µg/g fresh weight; (Fearing *et al.*, 1997)) compared with all other concentrations in the other diets (for more details see Hilbeck *et al.*, 1998a,b, 1999, Hilbeck, 2001).

Choice trials

The influence of transgenic Bt-corn plants on the prey preference of the predator *C. carnea*

Table 1. Summary data of bi- and tri-trophic feeding trials with *Chrysoperla carnea*. Mean total mortality of *C. carnea* larvae is presented.

	Bi-trophic Direct (toxin) (Hilbeck *et al.*, 1998b)	Tri-trophic Bt-corn (toxin) (Hilbeck *et al.*, 1998a)	Bt-incorporated diet (Hilbeck *et al.*, 1999)
Bt-concentration	100 µg/ml	ca. 4-5 µg/g fresh weight*	25, 50, 100 µg toxin /g diet
Bt-treatments	57% (only AD)[a]	59%[a] (*S. littoralis*)	55[a], 68[a,b], 78%[b] (Cry1Ab toxin) (*S. littoralis*)
Control(s)	30% (only AD)[b] 8% (*E.k.* only)[c]	37%[b] (*S. littoralis*)	26%[c] (*S. littoralis*)

[a,b,c] = Different letters between rows within columns represent treatment means that are significantly different at P=0.05 (LSMEANS); * Fearing *et al.*, 1997; AD = artificial diet only; *E.k.* = *Ephestia kuehniella* eggs only; *S. littoralis* = type of prey used in trials.

has been studied using paired-choice assays in a tri-trophic system (Meier & Hilbeck, 2001). Two different non-target prey species were used in the experiments; aphids (*Rhopalosiphum padi)* (Homoptera: Aphidae) and lepidopteran larvae (*Spodoptera littoralis)* (Lepidoptera:Noctuidae) raised either on Bt- expressing corn or the corresponding isogenic untransformed Bt-free corn hybrid. When *C. carnea* could choose between *S. littoralis* fed on transgenic Bt-corn and *S. littoralis* fed on non-transgenic corn, they showed a significant preference for *S. littoralis* fed on non-transgenic corn as 3rd instars. Although not statistically significant, a similar trend was observed for the 2nd instar. No preference was observed when *C. carnea* had the choice between *R. padi* fed on transgenic Bt-corn and *R. padi* fed on non-transgenic corn. This lack of preference for *R. padi* fed either transgenic or non-transgenic corn may be due to the absence of the Bt-protein in the phloem (Raps *et al.*, 2001). In prey combinations with *S. littoralis* and *R. padi,* the second and third larval stages of *C. carnea* showed a preference for *R. padi* regardless of whether they had fed on transgenic or non-transgenic corn. But *S. littoralis* still constituted 30 - 42% of the total amount of eaten prey of *C. carnea* larvae even in the presence of abundant aphids. In particular as first instars – presumably the stage most susceptible to Bt proteins – no statistically significant prey preference was observed, suggesting that *C. carnea* ate equal amounts of aphids and caterpillars in that stage (Meier & Hilbeck, 2001). Hence, even in a choice situation, *C. carnea* larvae were still exposed to the toxin *via* the consumed lepidopteran larvae.

RISK ANALYSIS AND POST-RELEASE MEASURES

Laboratory trials have identified that Bt proteins and Bt-plants can pose a hazard to *C. carnea* larvae. Region- and crop-specific analyses must now be carried out to estimate the risk of this hazard in the field. This requires careful examination of possible exposure routes in the affected agro ecosystem and a good working knowledge of the main herbivore species constituting the prey spectrum of the natural enemy. For green lacewings, this prey spectrum can be large because of the distinct polyphagy of this species (Bay *et al.*, 1993, Canard, 2001, Principi & Canard, 1984). In the following section, a brief risk analysis will be carried out,

and further research needs for a more comprehensive analysis will be identified.

If aphids are abundant in a transgenic Bt-crop field, our results suggest that *C. carnea* would probably feed preferentially on the aphids. In separate investigations on the same corn varieties used in these trials, we found no Bt proteins in the phloem sap or in the aphids feeding on the corn (Raps *et al.*, 2001). Hence, aphids are not likely to be posing a hazard to *C. carnea* larvae. However, our choice-feeding trial data suggest that *C. carnea* larvae will not feed exclusively on aphids as long as other prey species are also present in the agroecosystem. So in a typical, multi-herbivore species field situation, *C.carnea* must be expected to be exposed to the toxin at least at low levels even when aphids are dominant. In the absence or at low densities of aphids, the composition of the herbivore community present in the system will probably determine whether *C. carnea* is adversely affected. No data is available to date on the minimum Bt-protein uptake and exposure necessary to induce adverse effects in the *C. carnea* larvae. Non-exposure to the Bt-toxin will only be likely in a no-choice situation where *C. carnea* has only aphids available as prey. However, this situation will rarely occur or only be of short duration during a population peak of aphids.

Switching to Bt-free prey (phloem feeders) where they are present may be an example of a mechanism by which *C. carnea* can avoid the detrimental effects observed in the no-choice trials (Hilbeck *et al.*, 1998a, 1998b & 1999). For the Bt-corn, this preferential feeding behaviour could possibly lead to an increased predation pressure on aphids but, simultaneously, to a reduced predation pressure for Bt-containing prey. These findings may therefore also have implications for pest resistance development. As has been demonstrated in models by Gould *et al.*, (1991), natural enemies can either increase or decrease the rate of adaptation to the Bt protein. The models have so far considered differing degrees of susceptibility of the pest species, functional response types of the natural enemies and pest density dependent or independent predation behaviour, but not selective feeding behaviour of predators or adverse effects of the novel compound on the natural enemy.

In an on-going project by three research groups (ETH Zurich, Switzerland; Agrobios, Metaponto, Italy; Gödöllö University, Hungary) studying the ecological implications of Bt crop plants in the field, we identified the following relevant trophic relationships in Bt-corn, Bt-eggplant and Bt-potato cropping systems in Hungary and Italy, respectively (Table 2). In the Bt-corn fields in Hungary during the first year field trials, roughly 17 herbivore species and 30 natural enemy species were identified. In Bt-potato and Bt-eggplant plots in Italy, over 12 different herbivore and more than 14 different insect natural enemy species plus 5 different arachnid species were recorded. Some of the more important exposure routes for different natural enemies are listed in Table 2. Although still under evaluation, the abundance data shows that on Bt-corn in Hungary, aphids, spider mites and chrysomelid beetles are the most abundant prey species for *C. carnea*, while on Bt-eggplants and Bt-potatoes in southern Italy, cicadellidae, white flies, mites and thrips constitute a significant portion of the available prey spectrum. Region-specific information about co-occurring population dynamics and abundances of both the main prey and *C. carnea* will provide rough estimates of expected exposure levels and the resulting risk for *C. carnea* in those cropping systems. This risk will be different in Hungary and in southern Italy, i.e. aphids as Bt-free prey alternative are much less available in the Italian cropping systems than in the Hungarian one. Further research is needed to determine the minimum uptake necessary to induce adverse effects and to establish the hazard other Bt-fed non-target prey can pose to *C. carnea*.

Table 2. Food web components in Bt-crop fields and plots in Hungary and Italy (first year preliminary field results)

Transgenic Plant (*target herbivore*)	Country of release	Non-target herbivores = prey	Non-target natural enemies	NE preferred feeding habit
Cry1Ab potato (*Phthorimaea* spp.)	Southern Italy	***Leptinotarsa decemlineata* (Colorado potato beetle)**	***Chrysoperla* spp.**	**polyphagous**
			Miridae (*Orius* spp.)	Thrips
			Nabis spp.	
		Lygeidae spp.		polyphagous
			5 spider species	
		Thripidae spp.		polyphagous
		2 Cicadellidae spp.		
		2 Aphididae spp.		
		Aleyrodina spp.		
		Psyllidae spp.		
		Lyriomyza spp.		
Cry3B egg plant (*Leptinotarsa decemlineata*)	Southern Italy	**Spider mites**	***Chrysoperla* spp.**	**polyphagous**
		Phthorimaea spp.	*Stethorus* spp.	Spider mites
		Lygeidae spp.	Miridae (*Orius* spp.)	Thrips
		Thripidae spp.		
		2 Cicadellidae spp.	4 spider species	
		1 Aphididae spp.		polyphagous
		Aleyrodina spp.		
		Psyllidae spp.		
		Lyriomyza spp.		
Cry1Ab corn (*Ostrinia nubilalis, Helicoverpa armigera*)	Hungary	*Helicoverpa armigera* (T?)	***Chrysoperla* spp.**	**polyphagous**
		Diabrotica virgifera	12 Coccinellidae spp	oligophagous (aphids or spider mites)
		Spider mites	*Forficularia auricularia*	Aphids
		5 Aphididae spp.	*3 Orius spp.*	Thrips
		5 Chrysomelidae spp.	*Nabis spp.*	polyphagous
		Thripidae spp.	Carabidae *spp.*	polyphagous
			2 parasitoid *spp.*	host-specific
			Spider species	polyphagous

Most abundant herbivore prey for *Chrysoperla* spp. are printed in bold

CONCLUSIONS

If transgenic crops for crop protection are to become an integral component of IPM systems, comprehensive plans for their incorporation have to be developed and communicated to the farmers prior to their commercial release. This could include modified scouting and monitoring schemes for non-target pests/weeds and their natural enemies, and non-target pest/weed control methods that comply with IPM guidelines. Thresholds developed in conventional varieties may not be valid in Bt- or HT-crop fields.

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Transgenic papaya: a case for worldwide control of papaya ringspot virus

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ABSTRACT

Papaya ringspot virus (PRSV) was detected in the main papaya growing region of Hawaii in 1992. By 1994 Hawaii's papaya industry was facing devastating damage from PRSV. Efforts to develop resistant transgenic papaya were started in the mid 1980s. By 1991, a resistant line was identified, field trialed, and subsequently released to growers in 1998. 'Rainbow' an F_1 hybrid from a cross of the transgenic 'SunUp' and non-transgenic 'Kapoho' is now widely planted and has virtually saved the papaya industry in Hawaii. Other transgenic papayas have been produced for other countries and our data suggest that worldwide control of PRSV by transgenic papaya is possible. The technical and regulatory hurdles that had to be overcome in Hawaii will be discussed, along with our approach for worldwide control of PRSV.

INTRODUCTION

Papaya (*Carica papaya*) is a large herbaceous plant that is widely grown in the lowland tropics in large plantations and as a garden plant. Hawaii has produced papaya for over a century and is well known for its production of the 'Hawaiian solo' type papaya, which is smaller but generally sweeter than the traditional large fruit that is grown in many parts of the tropical world. The first report of *Papaya ringspot virus* (PRSV) was in Hawaii in the 1940s. Since then an abundance of reports have clearly established PRSV as the most widespread and severe viral disease of papaya. PRSV is a potyvirus that is rapidly transmitted by a number of aphid species in a non-persistent manner. The virus is grouped into two biotypes; PRSV-p infects cucurbits and papaya, while PRSV-w infects cucurbits but not papaya. The severity of PRSV world-wide is due to its rapid spread by insects and the lack of resistance in *C. papaya*.

The seminal report (Powell-Abel, *et al.*, 1986) on the resistance or tolerance of transgenic tobacco expressing the coat protein gene of tobacco mosaic virus spurred numerous laboratories to follow this approach for other viruses and crops. We started work towards the development of transgenic papaya in 1986. Our goal was to control PRSV in papaya in Hawaii, even though PRSV was only of minor economic importance at that time. However, in 1992 the invasion of PRSV into the Puna district of Hawaii island, where nearly all of Hawaii's papaya was being grown, put Hawaii's papaya industry into a crisis situation and threatened its survival. Ironically, we had just started a field trial of transgenic papaya in Hawaii in 1992. This communication briefly describes our work to develop and bring the transgenic papaya to commercialization and help save the Hawaiian papaya industry. It also describes our work towards the world-wide control of PRSV.

PAPAYA AND PRSV IN HAWAII BEFORE THE CRISIS

Commercial papaya was largely grown on Oahu island until the 1950s. Even though PRSV was discovered on Oahu island in the 1940s, it did not cause severe damage to the papaya crop until the late 1950s when, apparently, a new more severe strain of PRSV was discovered. By the late 1950s, PRSV had devastated the papaya industry on Oahu island, causing the industry to relocate to the Puna district of Hawaii island. Several factors caused Puna to become dominant in papaya production. These were the lack of PRSV, lots of sunshine and yet high rainfall, lots of available land that could be leased inexpensively, and the fact that papaya thrived in the volcanic lava land. In fact, the papaya acreage for the state of Hawaii increased from 540 in 1957 to 2,415 in 1992, of which 95% was in Puna by 1992.

However, PRSV was a potential threat to papaya grown in Puna because PRSV was present in the garden plantings of households in Hilo, a city located about 19 miles away from Puna. The Hawaii Department of Agriculture recognized the threat and deployed surveillance teams that constantly looked for and removed PRSV infected trees in Hilo and the nearby areas. Research was started in 1978 to develop control measures for PRSV in Hawaii. We initially worked on developing a mild strain of PRSV for use in cross protection. Although the mild strain provided protection to PRSV in Hawaii, it also caused significant symptoms on the Hawaiian solo papaya and thus was not used as a routine control measure for PRSV.

DEVELOPMENT OF TRANSGENIC PAPAYA: A MATTER OF TIMING

The early reports on developing virus resistant transgenic tobacco spurred us to start a program in 1986 to develop transgenic papaya for controlling PRSV. The team consisted of Jerry Slightom from Upjohn, Richard Manshardt from the University of Hawaii, Maureen Fitch a graduate student of Richard Manshardt, and myself. We pursued the strategy of transforming papaya with the coat protein gene of a mild nitrous acid mutant of a severe PRSV strain from Hawaii (the severe strain is designated as PRSV HA while the mild strain is PRSV HA 5-1.). Jerry Slightom led the engineering of the coat protein gene; Maureen Fitch led the transformation work; Richard Manshardt led the subsequent breeding; and I led the virology part. Since our goal was practical, we set about transforming the yellow-flesh Kapoho, which was exclusively grown in Puna, and Sunset, which was planted little in Hawaii but widely in Brazil. Transformation of somatic embryo cultures using a biolistic approach was started in 1988. Transformation and regeneration of Kapoho proved elusive, but we were able to obtain a limited number of transgenic lines of Sunset, and fortunately by 1991, one line (55-1) of Sunset showed resistance to PRSV HA in greenhouse inoculations.

A key decision in 1991 helped us deploy the transgenic papaya in a timely manner. Rather than waiting to get seeds from the R_0 plants of line 55-1 then testing them for resistance, we decided to test the resistance of R_0 plants of line 55-1 in field conditions using clonal cuttings of line 55-1 and non-transgenic Sunset as controls. The trial started in April 1992 on Oahu island. Coincidentally, PRSV was discovered in Puna on Hawaii island in May 1992.

PRSV spreads in Puna, creating a crisis for the industry

With Puna growing 95% of Hawaii's papaya, the potential devastation that PRSV could do to the industry was obvious. Immediate and large-scale actions were taken to suppress the

spread of PRSV in Puna. Initial massive cutting of trees and cooperative programs of tagging infected trees by state government officials followed by cutting of the trees by growers only slowed the inevitable spread. Thus, by October 1994 the virus was widespread and efforts to contain the virus were abandoned, causing an even faster spread of the virus. In an effort to keep production up in the state of Hawaii, new plantations were started on different areas of Hawaii island. Although these areas did not have the virus, the Kapoho variety did not adapt well to these regions. The result was that papaya production continued to drop and Hawaii began to lose market share in the mainland US.

Resistance of line 55-1 and development of transgenic cultivars

A major benefit of the 1992 R_0 field trial was that it allowed us to evaluate the resistance and the growth of the R_0 plants in replicated trials and helped us to develop cultivars that might be useful to the industry. Since Sunset and Kapoho breed 'true to type', growers normally get seeds from fruits of commercially grown trees. As mentioned earlier, the yellow-flesh Kapoho was the dominant cultivar in Hawaii, but line 55-1 was a red-flesh transgenic Sunset. The transgenic Sunset, which had a single insert of the coat protein gene, was brought to homozygosity for its coat protein gene and named 'SunUp'. However, growers in Hawaii prefer the yellow flesh type cultivar such as Kapoho. To develop a cultivar with yellow flesh, virus resistance and hopefully have commercially acceptable quality, an F_1 hybrid of transgenic 'SunUp' and non-transgenic Kapoho was created. This hybrid was named 'Rainbow'.

RACE TO COMMERCIALIZE THE TRANSGENIC PAPAYA

The race to commercialize the papaya was not to beat a rival competitive company but rather to stem the destruction of the papaya industry. In order for genetically engineered plant to be commercialized, it must be deregulated by various governmental agencies and licenses must be obtained from people or companies that hold the intellectual property rights to the components or processes that were used to create the transgenic plants. The regulatory agencies we dealt with were APHIS (Animal Plant Health Inspection Service), EPA (Environmental Protection Agency), and FDA (Food and Drug Administration). The intellectual property rights were held by several companies, including Monsanto.

In 1995, a large field trial was set up in a farm in Puna where PRSV had caused the farmer to abandon growing of papaya on the farm. The trial consisted of replicated blocks and a large solid block of Rainbow papaya to simulate commercial production and allow researchers, farmers, and packers to assess the quality, productivity, and acceptability of the fruit. The trial also helped us obtain data that were requested by regulators, such as on the spread of the transgene to border rows of the field trial and to papaya in abandoned fields that were far removed from the test site. The papaya crisis forced us to do activities concurrently, rather then sequentially as would have been done under noncrisis situation. For example, we started deregulation procedures even before we had much data on the quality of Rainbow and SunUp, and efforts to obtain licenses were started soon thereafter.

The field trial conclusively demonstrated that Rainbow and SunUp were resistant to PRSV under intense virus pressure. Data on the field trial were taken for two and half years. We did not observe resistance breakdown in the test transgenic trees. The yield and quality of

Rainbow were exceptional, amounting to an annual yield of 125,000 pounds of papaya per acre, infected non-transgenic controls yielded only5,000 pounds per acre.

Efforts to deregulate the papaya proceeded in a timely manner and APHIS deregulated it in November 1996, EPA in August 1997 and FDA completed its consultation in September 1997. It should be mentioned that our efforts in developing the transgenic papaya, testing them in the field, and deregulating the papaya were transparent and for the sole purpose of moving as prudently as possible towards evaluating and eventually releasing a product to help save the papaya industry. We did not experience public protests or demonstrations over the work. The task of obtaining the licenses for the components of the papaya that were covered by patents were turned over to the PAC (Papaya Administrative Committee), which is composed of papaya growers who have organized themselves under a USDA marketing order, and who pay an assessment fee for each pound of papaya that they sell. Fortunately, these efforts also went well and the necessary licenses were obtained by the PAC by April 1998.

RECLAMATION OF PUNA AND IMPACT OF TRANSGENIC PAPAYA

A celebration to signal the debut of the transgenic papaya was held on May 1, 1998 and transgenic papaya seeds were distributed free on the same day. Distribution was done by a lottery system because the quantity of seeds was limited. Rainbow comprised the overwhelming amount of the distributed seeds. Much of the seeds were quickly planted and by late 1998, many previously abandoned fields were being reclaimed and new sites being planted. The decline of the papaya industry had been halted by the transgenic papaya. The transgenic papaya showed excellent resistance, even when planted next to heavily infected fields. Harvesting of Rainbow was started in 1999, and grower, packer, and consumer acceptance were widespread. The following production statistics bear out the impact of the virus on production and the impact of the transgenic papaya on increasing the production of papaya. In 1992 when PRSV was detected in Puna, the area produced 53 million pounds of fresh marketable papaya. PRSV caused a steady decline in production such that Puna produced only 26.7 million pounds in 1998, the year the seeds were released. Production started to rebound and in 2001, Puna produced 40 million pounds of marketable papaya.

As mentioned earlier, PRSV had previously eliminated papaya production on Oahu island. The transgenic papaya has revived commercial production on Oahu island. The island now grows Rainbow and other hybrids that have been created by crossing non-transgenic papaya lines with Rainbow.

TOWARDS THE WORLDWIDE CONTROL OF PRSV

The initial success of the transgenic papaya in Hawaii spurred us to implement this technology in other countries. My laboratory was contacted by various countries to collaborate in developing transgenic papaya for their specific regions. The common problem in all these countries was lack of resistance to PRSV. We established a generalized approach that was cost effective and could lead to sustainable transfer of technology. Basically, a scientist or a graduate student came to my laboratory for the specific purpose of developing the transgenic papaya, testing the transformants for resistance, and transferring the papaya

back to their country. We would then collaborate to move the transgenic papaya through the testing and commercialization process of the target country.

Our goal was to engineer the genes, transform, and initially test transformants in about 18 months if a scientist came to the lab. For graduate students, the process took longer because the work became part of their thesis. Coat protein genes from PRSV isolates found commonly in the respective countries were engineered, desired cultivars were transformed, and initial tests were done against virus isolates from the target countries. Cornell was an ideal place to do the work. We had collected a large array of PRSV isolates from around the world and since papaya is not grown commercially in New York, PRSV strains could be introduced for greenhouse work without danger of harming a papaya industry. Plus, the technical and intellectual property expertise was readily available. Resistant transgenic papaya have been developed and transferred to Brazil, Jamaica, Venezuela, and Thailand. Field trials have been established in Jamaica and Thailand.

IMPACT OF THE GMO CONTROVERSY ON TECHNOLOGY TRANSFER

Hawaiian case

Our efforts to develop and commercialize transgenic papaya for Hawaii did not meet any significant resistance. Consumers have accepted the transgenic papaya in the US. So Europeans might ask, "why didn't you get resistance and public protests against the work you were doing?" Some reasons are that we were simply scientists that were trying to help an industry that was in a crisis, we were not supported by companies, we kept the people of Hawaii well informed of our work, there are good arguments that transgenic plants that express coat protein genes of plant viruses are not dangerous to human health or the environment, wild species of papaya do not exist in natural habitats of Hawaii, papaya is not an important commodity crop, and papaya was commercialized before the momentum of the GMO controversy built up. All of these reasons probably contributed to the general acceptance of the work.

Transgenic papaya in other countries

The transgenic papaya that we developed for Brazil, Jamaica, Thailand, and Venezuela are at various stages of testing and deregulation. The transgenic papayas are showing good resistance and horticultural properties. The deregulation process is furthest developed in Jamaica and Thailand. We are working with personnel in these countries on providing the required technical data and sharing of our experience from the Hawaiian case.

IMPROVEMENT OF TECHNOLOGY

Our research has shown that transgenic resistance is RNA mediated and thus homology dependent. The resistance of SunUp and Rainbow are affected by transgene dosage, plant development stage, and coat protein sequence homology of the attacking strain. SunUp, which is homozygous for the CP gene of PRSV HA 5-1, is resistant to the Hawaiian isolates and many isolates outside of Hawaii. Whereas, Rainbow, being hemizygous for the CP transgene, is resistant to Hawaiian isolates but susceptible to most isolates outside of Hawaii.

The practical implication of these observations is that a single CP gene will not impart resistance to a broad range of PRSV isolates. To develop resistance to a broader range of PRSV isolates, we have transformed plants with a transgene that consist of short linked segments (ca 250 nucleotides) of coat protein genes from several PRSV strains. Our recent data show that these transgenic papaya do show resistance to multiple strains of PRSV. Lastly, we have recently developed 'synthetic' genes that might provide multiple resistance. These advancements should help us in our quest to have worldwide control of PRSV.

SOME CONCLUDING THOUGHTS

The Hawaiian papaya story describes a rather fortunate case where technology development came in a timely manner to help save the papaya industry. It also represents a clear example of the power and durability of resistance in plants that express viral genes. It is rather surprising to me that the technology has not been exploited to control a myriad of viral diseases. Are we looking too closely at the negative aspects of this technology? A number of people have said that the Hawaiian papaya case is an ideal scenario because there was 'no other alternative.' Do we have to have such extreme cases before this technology of virus resistance is deployed? I don't think so. The current mode of transgenic virus resistance is simply an approach that has been developed because of our drive to control viral diseases. We should take advantage of these current technologies to meet our primary goal as plant pathologists.

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Adaptive resistance management in *Bt* maize

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ABSTRACT

Insect resistance management (IRM) should comprise an initial IRM plan, monitoring methods to provide information on the progress of resistance evolution, and a response strategy to modify IRM as evolution proceeds. For *Bt* maize in the US the initial IRM plan is the high-dose/refuge plan. Several key biological parameters still need to be estimated before the scientific assumptions underlying this strategy can be verified, including *R* allele frequency in European corn borer (ECB) in the southern corn belt of the US and in southwestern corn borer, the rate of female and male dispersal from natal fields and management interventions that can modify these rates, dominance of resistance, and the occurrence of developmental delays associated with resistance. The F_2 screen is a cost-effective, sensitive method for monitoring *R* allele frequencies in ECB so that IRM for *Bt* maize can be modified as evolution proceeds. This procedure is constantly being improved, but additional research is needed to determine where to sample for resistance. The evidence is sufficient to imply that an adaptive response strategy could provide many more years of efficacy for these insecticidal crops, but additional research is needed to justify a response strategy. IRM is not yet a mature science, and many scientific questions have only recently been framed. It seems essential that flexibility be incorporated within an IRM plan to enable it to be adapted as new information accumulates.

INTRODUCTION

Bt maize is a group of maize varieties that is genetically engineered to express a crystal protein gene from the soil bacterium, *Bacillus thuringiensis*. These crystal protein genes are called *cry* genes, and the proteins they produce are called Cry proteins. There are several hundred *cry* genes that have been found in naturally occurring *B. thuringiensis*, but relatively few have been used to make *Bt* maize. Presently, *Bt* maize relies only on the Cry toxins that are toxic to moths and butterflies. The maize varieties are distinguished by the transformation event used to introduce the *cry* gene into maize. Of the thousands of transformation events that may occur during the development of a single *Bt* maize variety, only one is developed commercially. In total, the products from 6 different transformation events have been commercially sold in the US as *Bt* maize, of which only 3 of remain on the commercial market at this time (Bt-11, Mon 810, and TC 1507). Bt-11 and Mon 810 are Cry1Ab toxins and TC1507 is a Cry1F toxin. The commercially available *Bt* maize varieties all are very effective at controlling ECB, *Ostrinia nubilalis* (Hübner). Survival rates of susceptible ECB on these varieties have not been measured definitively, but probably they are all <0.001. Of course, survival in fields *of Bt* maize will be significantly higher because some of the plants in every field do not express Cry toxin because the seed production process does not assure 100% seed purity.

All experts agree that ECB will evolve resistance to *Bt* maize at some time in the future. The

US Environmental Protection Agency (EPA) has decided that some form of insect resistance management (IRM) must be implemented to delay this inevitability for at least 15 years, and if possible even longer. I will review the status of resistance management for *Bt* maize, and suggest that an adaptive management strategy should be developed and adopted.

AN ADAPTIVE MODEL FOR IRM

All management systems rely on monitoring to adjust practices as conditions and situations change. This adjustment process is called adaptive management, and it should form the basis for IRM (Figure 1). An initial IRM plan should be established prior to the initial commercialization of the *Bt* maize. This IRM plan should be based on the best scientific information available. In addition, a system to monitor the development of resistance should be established so that control failures can be anticipated. From the monitoring information, it will be possible to identify regions where the risk of control failure has substantially increased. In these regions, IRM practices can be adapted to further delay the evolution of resistance. This comprehensive system has not yet been developed for any *Bt* crop. The initial plan has been developed and is presently being implemented for *Bt* maize. The monitoring system needs additional scientific research to justify their use in a cost-effective manner. Relatively little scientific research has been done on how to revise the management plan.

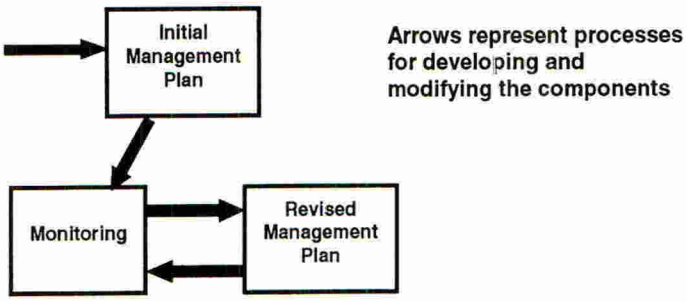

Figure 1. Schematic of adaptive insect resistance management.

INITIAL MANAGEMENT PLAN

The initial IRM plan for *Bt* maize is a 20% structured refuge, as required by the US-EPA. This means that 20% of the maize in an area must be non-*Bt* maize, which acts as a refuge for ECB. This refuge must be within ½ mile of *Bt* maize, and in this sense it is structured on the agricultural landscape. The scientific evidence supporting this IRM strategy is summarized in theoretical mathematical models, which encompass vast amounts of biological information. These models are essential for determining appropriate resistance management practices. In addition to integrating the best available biological knowledge, these models also lay bare the biological assumptions that enter into the analysis of resistance risk and its management. For example, two common assumptions in most models of resistance evolution are (1) that evolution is driven by directional selection, and (2) there is no cost to resistance. The first assumption indicates that the fitness of the *RS* heterozygotes is intermediate between the fitness of the *SS* and *RR* homozygotes (*R* is a resistance allele and *S* is a

susceptible allele). This means that heterosis cannot maintain intermediate levels of resistance. The second assumption is that insects with resistance to *Bt* maize (*RR* genotypes) have the same fitness as *SS* genotypes when feeding on non-*Bt* maize. This assumption is not entirely realistic biologically. Most cases of insecticide resistance have costs associated with the resistant phenotype. However, this cost has been shown to evolve to nothing in the few cases where this has been examined. These two assumptions specify a reasonable worst-case scenario, which is a useful, precautionary benchmark from which to evaluate the potential utility of an IRM strategy. One of the main consequences of these assumptions is that resistance cannot be prevented; it can only be delayed. Because delaying resistance is more readily accomplished than preventing resistance, these assumptions have the distinction of both being precautionary with respect to risk and at the same time more easily attained for applicants and industry.

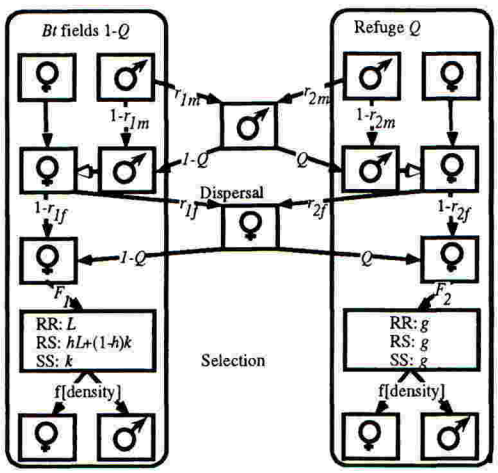

Figure 2. Schematic of evolution model for insect resistance to a *Bt* crop, based on Comins (1977) model and analyzed by Ives & Andow (unpublished).

Most models of the evolutionary process have at their core some variant of a model first developed by Hugh Comins, who modeled the problem of resistance evolution to insecticides (Comins, 1977). Diagrammatically, one such model is depicted in Figure 2. There are two field types, *Bt* and refuge, with proportional areas of 1-Q and Q respectively. Some males move before mating, and some females move after mating, and oviposition occurs after moving. Subsequent larvae are exposed to selection in the *Bt* fields according to their genotype, while those in the refuge may be killed by insecticides with efficacy 1-g. Dominance is given by the parameter h, with $h = 0$ for a completely recessive resistance allele and $h = 1$ for a completely dominant one. Populations of larvae then undergo density-dependent mortality, and the resulting adults repeat the cycle.

Many simulations can be run from these kinds of models, and a typical result is shown in Figure 3 (data from Alstad & Andow, 1995). Using parameter values that are realistic for ECB and an initial R allele frequency of 0.003, which is quite high, the effects of a 20% refuge under high dose conditions can be modeled. Without the refuge, resistance can evolve in as little as 3 generations. In this simulated example, resistance is delayed to 14

generations, about 5 times longer than when no refuge is used. Thus, models suggest that the high-dose refuge strategy can work to delay resistance.

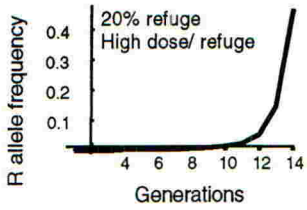

Figure 3. Possible outcome of resistance evolution using a refuge.

High dose

High dose means that resistance in the insect is nearly completely recessive. Hence, *RS* heterozygotes suffer similar mortality as *SS* homozygotes when feeding on *Bt* maize and h, the dominance parameter must be near 0. Experts accept $h < 0.01$ for BT-11 and Mon 810, which would imply that they are both probably high dose events. Because h can be measured only after resistance has been recovered from natural populations, the initial management plan for *Bt* maize is based on scientific guesses about the value of h; nearly all scientific information available to date suggests that resistance to *Bt* maize is likely to be recessive.

Refuge

A refuge is a habitat where *SS* homozygotes have similar fitness as *RS* and *RR* genotypes. In theory, a refuge could be any habitat where the pest occurred, including non-*Bt* maize, non-maize crops, and other non-crop plants. For ECB and *Bt* maize, these non-maize habitats do not appear to be productive enough to maintain viable populations of ECB, so the refuge is required to be non-*Bt* maize. A similar result has been found for non-crop hosts of corn rootworms, so the IRM strategy for these species also is likely to rely on non-*Bt* maize refuges. For resistance management, a refuge creates spatially variable selection, which acts to delay resistance. A 20% refuge is required for present *Bt* maize varieties in the US by the EPA.

Structured refuge

A refuge is structured when its spatial location with respect to the *Bt* crop is constrained by some requirement. The reason the refuge is structured is to ensure that it is close enough to the *Bt* crop so that it can function as a refuge. Without such a constraint, many cotton farmers in the US have chosen to plant their refuges on the poorer soils several miles from their most productive land. When this happens, there is no effective refuge, and the management plan will fail. For *Bt* maize, the refuge must be within ½ mile of *Bt* maize. Unfortunately, this specification does allow for large contiguous blocks of fields of *Bt* maize to be planted. If the requirement had been that all *Bt* maize must have a refuge within ½ mile, such blocks would not be possible.

How does the high-dose/refuge strategy work?

There are several explanations for how the high-dose/refuge strategy works to delay

resistance. In some recent research conducted by A R Ives & D A Andow (unpublished), a class of high dose events called 'high-efficacy high-dose events' is identified. These events are assumed to have a low *h*, like all high-dose events, but also have high *SS* mortality in *Bt* fields. The results of our analysis of this case apply to Bt-11 and Mon 810 *Bt*-maize against ECB and southwestern corn borer, *Bt*-cotton against cotton bollworm, and probably the Pioneer-Dow binary toxin against corn rootworms. The analysis does not apply to Mon 863, the Cry3Bb corn rootworm event or *Bt* cotton in Australia or east Asia. For small *k* events, we found that the rate of resistance evolution depended only on the proportion of *SS* homozygotes exposed to *Bt* toxin and the fitness of the *RR* and *RS* genotypes in the *Bt* field. The evolutionary rate did not depend on the productivity of the refuge, beyond the requirement that the refuge is capable of maintaining a viable population. This analysis leads to several significant and controversial conclusions (Ives & Andow, unpublished).

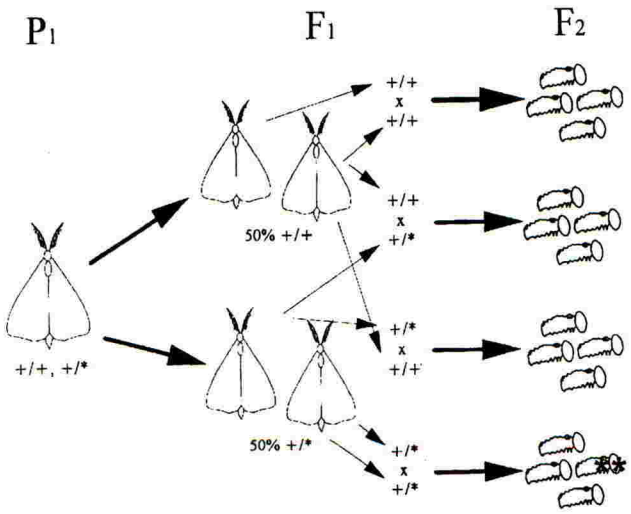

Figure 4. Schematic of an F_2 screen. A mated female is caught from a natural population (P_1), and her family is reared separately and allowed to mate with siblings. With random sib-mating, 1/16 of the F_2 offspring are expected to be homozygous for any * allele carried by the female, either in her own genome or her mate's genome.

How common is resistance?

Resistance must be rare enough for IRM to be effective. If it is too common, then control failures will occur rapidly and no IRM strategy is likely to be effective at delaying resistance evolution. For the high-dose/refuge case, the resistance allele frequency should be <0.001 for a significant delay in resistance evolution. However, estimating an allele frequency of 0.001 for a recessive allele in a natural population is a logistical challenge. For a totally recessive trait occurring at a frequency of 0.001, only one in a million individuals will have a resistant phenotype (0.001^2). This means that over one million individuals from a natural population must be screened in order to find even one resistant individual. This is impossible logistically for any diploid species.

More efficient methods have been developed that improve precision and reduce the effort to more manageable levels (Andow & Ives, 2002). The most sensitive of these methods is the F_2 screen (Figure 4, Andow & Alstad, 1998). Because a mated female contains 4 haplotypes, and inbreeding during the F_1 generation concentrates recessive alleles into homozygous genotypes, this method is extremely efficient. For example, only 250 females is needed to detect a recessive allele at frequency 0.001. Unpublished results using the F_2 screen to estimate *R* allele frequencies in two populations of ECB indicate that the frequency is rare enough in those two populations that the high-dose/refuge strategy should work.

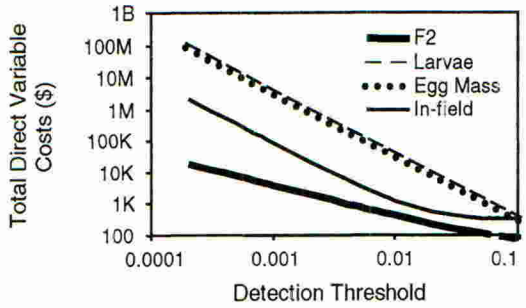

Figure 5. Relative cost of monitoring for a recessive allele as a function of the detection threshold that is the lowest detectable allele frequency.

The cost of an F_2 screen is significantly less than the cost of any other monitoring method, except perhaps molecular methods, for any given detection threshold for a recessive allele (Figure 5, data from Andow & Ives, 2002). Fixed costs, such as capital equipment and amortization are not included in these cost estimates. The variable costs for conducting an F_2 screen with a detection threshold of 0.001 amount to about $5000 for ECB. Thus it is probably feasible to use an F_2 screen to monitor resistance in ECB.

Unresolved scientific issues

Several scientific issues that are critical to the design of IRM remain unresolved. Resistance frequencies for ECB need to be measured in the south-central US and most of Europe, and resistance in southwestern corn borer still needs to be measured. Our evolutionary models suggest that several aspects of moth movement will be critical for determining the rate of resistance evolution, and none of these is adequately understood. Pre-mating *versus* post-mating female movement and male mate-finding movement is poorly understood in most pest insects, including ECB, yet these can have considerable effect on the rate of evolution. Finally, there are two key scientific issues that will only be resolved when resistance is recovered from natural populations, (1) the dominance of resistance, which is the key parameter influencing resistance evolution, and (2) the timing of emergence of the three genotypes (*RR*, *RS*, and *SS*), which will influence the probability of local positive assortative mating.

ADAPTIVE MANAGEMENT: MONITORING AND RESPONSE

Adaptive management couples monitoring with a response. It is most useful when there is sufficient uncertainty or when conditions change with time, and it is an effective way of

managing uncertainty or unexpected events. Because the initial resistance management plan has many scientific assumptions that cannot be confirmed, an adaptive approach could be useful.

Purpose of monitoring

There are several purposes for resistance monitoring. In adaptive management, the purpose of monitoring is to collect timely information that would allow a change in management that furthers the goals of management. This approach of using timely information to adjust management practices is at the core of Integrated Pest Management (IPM), so an IRM plan that is consistent with IPM will by necessity have an adaptive management component. In IRM, this would involve monitoring *R* allele frequencies so that the initial IRM plan could be improved when resistance is actually detected and recovered, long before there are control or management failures. Monitoring *R* allele frequency is necessary for an adaptive IRM plan. As discussed by Andow & Ives (2002), this can be done using an F_2 screen on ECB.

Several other purposes of monitoring have also been proposed, and it is critical that these purposes be clearly distinguished from each other because they will lead to differing monitoring and response systems. For example, monitoring has been proposed to document field failure of the IRM plan. It is certainly necessary to know when there has been a failure, but such knowledge does not help to improve the situation. The only alternative at that stage is to abandon the technology and substitute another, joining the pesticide treadmill, a consequence most entomologists would prefer to avoid.

Revising management

There are several fundamentally different ways to respond and adapt the high-dose/refuge plan when resistance is detected (Andow & Ives, 2002): killing insects in the *Bt* field, increasing the size of the refuge, and modifying movement rates of males and females. The *R* allele is selected and increases because it survives better than the *S* allele in the *Bt* fields. By killing pests in the *Bt* field, the selective advantage of the *R* allele can be reduced, thereby delaying resistance evolution. This might be done by spraying the *Bt* field with a non-*Bt* insecticide, autumn plowing of the *Bt* field to kill over-wintering *R* types, and so on. The refuge reduces the rate of resistance evolution in proportion to its size. Hence, another approach to adapting IRM would be to increase the size of the refuge. Analysis of these two approaches shows they provide modest delays in resistance evolution (Andow & Ives, 2002).

An entirely different approach is to modify movement rates of males and females from their natal fields (the field in which the adult emerges from it pupa). If resistance can be detected early enough (at frequencies between 0.001 and 0.004), and female and/or male dispersal can be changed, then resistance evolution can be delayed substantially (Figure 6, data from Andow & Ives, 2002). Thus, various approaches should be investigated to determine feasible and effective ways to adapt IRM to further delay resistance. In the results described here, it has not been shown that such efforts are efficacious, only that it is worth conducting experiments to determine the feasibility of managing male and female movement.

CONCLUSIONS

The initial IRM plan for *Bt* maize in the US has been developed using the best available

biological knowledge and mathematical models combining population genetics and population ecology. For *Bt* maize, this is the high-dose/refuge plan. Several key biological parameters still need to be estimated before the scientific assumptions underlying this strategy can be verified. These include *R* allele frequency in ECB in the southern corn belt of the US and in southwestern corn borer, the rate of female and male dispersal from their natal fields and management interventions that can modify these rates, dominance of resistance, and the occurrence of developmental delays associated with resistance.

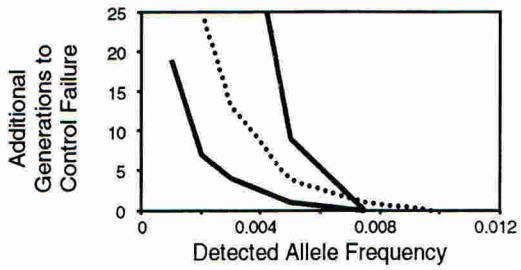

Figure 6. Additional number of generations to control failure compared to not changing IRM, when female dispersal is reduced and male dispersal is increased. Each line is a different combination of male and female movement parameters.

No effective monitoring plan for adapting IRM is presently being required by the US-EPA or being used voluntarily for any transgenic insecticidal crop. The F_2 screen is a cost-effective, sensitive method for monitoring *R* allele frequencies in ECB so that IRM for *Bt* maize can be modified as evolution proceeds. This procedure is constantly being improved, but additional research is needed to determine where to sample for resistance. No adaptive changes in IRM are being contemplated by US-EPA at this time. While the evidence is sufficient to imply that such adaptive changes could provide many more years of efficacy of these insecticidal crops, experiments to demonstrate their potential in the field have not yet been done.

IRM is not yet a mature science, and many scientific questions have only recently become evident. It seems essential that flexibility be incorporated within an IRM plan to enable it to be adapted as new information accumulates both about the scientific theory of IRM and the particular implementation associated with *Bt* maize.

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