

## ROLE OF OTHER NON-BT GENES IN MANAGING RESISTANCE TO BT CROPS

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### ABSTRACT

*Using the diamondback moth/Bt broccoli system as a model, we have investigated aspects important to the long-term deployment of this novel technology. Although the diamondback moth/Bt broccoli system may not exactly duplicate the currently available insect/Bt crop systems such as crops like corn, and potatoes, it can help identify areas for further work. Concurrently with more field studies conducted to refine the presently utilized recommendations, industry, public sector scientists, and farmers must work together to develop a second generation of technology and implementation strategies to ensure the even longer term durability of Bt-transgenic plants. Within the plant showed that toxin expression is fairly uniform throughout the plant when the plant is in its vegetative stage<sup>17</sup>. We used cytoplasmic male sterile transgenic plants hemizygous for the cryIA(c) gene for field tests. Additional treatments were included for reference to reflect native diamondback moth populations and were 0% refuge and 100% refuge with no insect release in either (one replicate each). Counts of larvae (all stages) were taken at five periods over the season beginning on 1 July and ending on 12 September. Leaf-dip bioassays with Javelin WG (Bt var. kurstaki; Novartis) to evaluate resistance were done with progeny of the released larvae (time zero) and with progeny of larvae counted in the final collection.*

**Keywords:** *Deployment, recommendations, industry, public sector*

### INTRODUCTION

Most people think that deciduous trees shed their leaves in order to avoid a winter, or a tropical dry season. And so they do. But this is not the only reason. They also shed their leaves to achieve a break in their parasitism, and to resuscitate their biochemical locks. This additional function of leaf-shedding explains several conundrums that baffled botanists for years. For example, it explains why a temporary resistance should evolve in a tree that lives for centuries. It also explains why a tree such as rubber (*Hevea brasiliensis*) should be deciduous, and have vertical resistance to a disease called leaf blight (*Microcyclus ulei*), even though it occurs wild in the Amazon valley, which is continuously warm and wet. And it explains why the members of the Mendelian school could not find any single-gene resistances in various important crops derived from wild plants that have continuous epidemics, such as sugarcane, citrus, and olives.

This, then, was the bane of Mendelian breeding for resistance. If a crop is derived from a wild plant that is an evergreen perennial, it will have horizontal resistance but no vertical resistance.

Conversely, if the wild progenitor of a crop is an annual herb, or a deciduous tree or shrub, that crop will have both horizontal and vertical resistances. The evolutionary survival value of a gene-for-gene relationship in a discontinuous epidemic is remarkable and, for this reason, it will often, but not necessarily, evolve in annual herbs, and against the leaf parasites of deciduous trees and shrubs. A Mendelian breeder, looking for a genetic source of qualitative, vertical resistance, will not find it in evergreen perennials. He may find it in crops with discontinuous epidemics, but he will not necessarily do so. A biometrician, on the other hand, looking for quantitative, horizontal resistance, will invariably find it, in any crop, and against any parasite of that crop.

It will be remembered that a Mendelian breeder needs a genetic source of resistance. If he cannot find it, the resistance breeding cannot even begin. A biometrician, on the other hand, does not need a genetic source of resistance. He needs merely to increase an existing level of quantitative resistance by changing gene frequencies in a mixed population. He can thus breed any crop for resistance to any parasite, and he can do so without first finding a source of resistance.

## REVIEW OF LITERATURE

We should note also that most of the crop species in temperate countries have discontinuous epidemics, and vertical resistances, because they evolved in a region that has winters. And most of the research in crop science has been done in temperate regions, and on temperate crops, grown in the wealthy, industrial nations. Conversely, many tropical crops have continuous epidemics, and they lack vertical resistance. But relatively little research has been done on these tropical crops, grown in impoverished, non-industrial countries.

These differences of climate and research have done much to exaggerate the importance of vertical resistance, and to disguise the importance of horizontal resistance.

Each year thousands of research hours and hundreds of thousands of research dollars are spent to battle caterpillars that love to eat crops. The pink bollworm, saltmarsh caterpillars, and beet armyworms have rapidly chewed their way through acres of crops, in some years totally decimating a crop. Crops growers fight to produce a salable product using chemical sprays, natural controls, cultural practices, pheromones (insect mating hormones) and monitoring.

After years of development, a completely new kind of tool is available for Arizona growers to use in warding off the pink bollworm, one of the state's major crops pests. Transgenic crops, a genetically engineered crops, carries its own insecticide within the plant tissues.

The scale of both the economic and environmental costs of insect control in agriculture, and of the losses incurred in spite of such measures, is high (Fig. 1). Therefore, it is not surprising that insect-resistant transgenic plants were among the first products of plant biotechnology to reach the marketplace. Recent commercial releases of genetically engineered crops have included transgenic corn, crops and potato, which express *Bacillus thuringiensis* (Bt) toxins (Box 1).

In North America, these crops are already being grown on a considerable proportion of the arable land. An even more diverse array of transgenic crops are scheduled for release in several countries. Here we review the use of Bt, the underlying scientific developments and the creation and introduction of transgenic Bt plants. The safety issues involved, and the concerns raised by the introduction of transgenic Bt plants into agricultural systems, are also discussed.

## MATERIAL AND METHOD

Currently the most promising ones being evaluated in transgenic plants include vegetative insecticidal proteins (vips), as well as various genes from other insects, animals, plants, and bacteria that act as inhibitors of insect digestive enzymes (e.g., protease inhibitors,  $\alpha$ -amylase inhibitors, and cholesterol oxidase). The development and implementation of engineered insecticidal plants is currently in its infancy and the only available technology is that of Bt-transgenic plants. Using the diamondback moth/Bt broccoli system as a model, we have investigated aspects important to the long-term deployment of this novel technology. Although the diamondback moth/Bt broccoli system may not exactly duplicate the currently available insect/Bt crop systems such as crops, corn, and potatoes, it can help identify areas for further work. Concurrently with more field studies conducted to refine the presently utilized recommendations, industry, public sector scientists, and farmers must work together to develop a second generation of technology and implementation strategies to ensure the even longer term durability of Bt-transgenic plants. Within the plant showed that toxin expression is fairly uniform throughout the plant when the plant is in its vegetative stage<sup>17</sup>. We used cytoplasmic male sterile transgenic plants hemizygous for the cry1A(c) gene for field tests. Additional treatments were included for reference to reflect native diamondback moth populations and were 0% refuge and 100% refuge with no insect release in either (one replicate each). Counts of larvae (all stages) were taken at five periods over the season beginning on 1 July and ending on 12 September. Leaf-dip bioassays with Javelin WG (Bt var. kurstaki; Novartis) to evaluate resistance were done with progeny of the released larvae (time zero) and with progeny of larvae counted in the final collection.

The answer appears to be that quantitative vertical resistance did not evolve to prevent allo-infection, or even to prevent parasitism. It evolved to prevent damaging population explosions, and it does this by controlling the reproduction of the parasite. And this is probably the ultimate function of all vertical resistances. A few infections, and a little damage to the host population, are quite unimportant compared with the disaster of an uncontrolled population explosion in the parasite.

We have seen that vertical resistances appear to reduce parasitism by reducing the frequency of matching allo-infection. And, at first sight, this reduction of parasitism appears to be the obvious function of vertical resistance. In fact, the ultimate function of vertical resistance is probably to reduce reproduction in the parasite and, hence, the control of population explosions in the parasite. Most vertical resistances achieve this by the simple expedient of controlling allo-

infection. A few do it by allowing allo-infection, allowing some parasitism, and some growth of the parasite, but by either preventing, or greatly reducing, parasite reproduction.

But this is a digression. Let us return to the two kinds of epidemic. In practice, this difference between continuous and discontinuous epidemics is crucial to the functioning of vertical resistance. Consider the epidemics of a leaf parasite of a hypothetical tree. If the tree is deciduous, the epidemic is discontinuous, and the vertical resistance will function at the start of every new epidemic. If the tree lives for, say, five hundred summers, its vertical resistance will protect it through five hundred epidemics. By chance, in a few of these epidemics, the tree will be matched quite early in the season, and it will suffer accordingly. However, every tree can tolerate an occasional bad epidemic. Equally, in a few of these epidemics, the tree will be matched so late in the season that it suffers no parasitism at all. On average, it will be matched sufficiently late for the parasite to do only very minor damage in each season.

Now consider an evergreen tree which has a continuous epidemic. Its first infection must be an allo-infection but, after that, it can remain parasitised by auto-infection for the rest of its life, and all auto-infection is matching infection. Vertical resistance would protect this evergreen tree only until the first matching allo-infection occurred, probably when the tree was still a very young seedling. The vertical resistance would then be useless for nearly five hundred subsequent summers. A gene-for-gene relationship cannot function in a continuous epidemic and, consequently, its evolutionary survival advantage is negligible. For this reason, a gene-for-gene relationship never evolves in host-parasite systems that have continuous epidemics.

**Bacillus thuringiensis:** a natural insecticide *Bacillus thuringiensis* is a gram-positive, spore-forming bacterium that exists in many locations, such as the soil, plant surfaces and in grain storage dust. *B. thuringiensis* can be distinguished from related

*Bacillus* species by the presence of parasporal crystals that are formed during sporulation. The parasporal crystals consist of one or more d-endotoxins or crystal (Cry) proteins of ~130 kDa (although truncated forms also occur). It is these that makes Bt an effective insect pathogen. Following ingestion, the alkaline environment of the insect midgut causes the crystals to dissolve and release their constituent protoxins. The protoxins are subsequently trimmed by gut proteases to an N-terminal, 65–70 kDa truncated form – the activated toxin (Fig. 2). The toxin binds to specific receptors on the cell membranes of the midgut epithelial cells, inserts itself into the membrane, and forms pores that kill the epithelial cells (and eventually the insect) by colloid osmotic lysis<sup>1</sup>. *B. thuringiensis* d-endotoxins are part of a large and still growing family of homologous proteins – more than 130 genes have been identified to date (see Bt toxin nomenclature at: [http://epunix.biols.susx.ac.uk/Home/Neil\\_Crickmore/Bt/index.html](http://epunix.biols.susx.ac.uk/Home/Neil_Crickmore/Bt/index.html)). These genes generate a rich source of diversity on which to draw for differing insect specificities. This specificity is an important aspect of the Bt Cry proteins: each protein is active in only one or a few insect species. Specificity is to a large extent determined by the toxin–receptor interaction<sup>2</sup>, although solubility of the crystal and protease activation also play a role. The members of the Cry gene family are grouped in subfamilies according to their specificity for members of the

insect families Lepidoptera (caterpillars), Diptera (flies and mosquitoes) and Coleoptera (beetles) 3. Some Bt strains have also been reported to be active against other insect families and also mites, nematodes, flatworms, and protozoa, but few details as to their practical use are available<sup>4</sup>. It is also significant that several important insect pests appear to be insensitive to known Cry proteins (for example, the Corn root worm, aphids, and white flies).

## CONCLUSION

*Bacillus thuringiensis* formulations (spore and crystal mixtures) were used as insecticidal sprays in the 1930s, but large scale production only started with the introduction of Thuricide<sup>®</sup> in the late 1950s, and this was followed by similar products from several companies<sup>5</sup>. In spite of their environmentally friendly reputation, Bt sprays have never occupied a large share of the insecticide market, and are largely used by organic farmers and gardeners and in forestry. Three factors are responsible for this:

- Lack of stability.
- Failure to penetrate tissues, and therefore to reach insects in all parts of the plant.
- Too narrow a specificity.

Crystal proteins degrade rapidly in UV light, losing their activity.

It is therefore necessary to make multiple applications throughout the growing season, which raises the costs of pesticide treatment.

Although some improvements have been made in this area, it remains the biggest single drawback to the use of Bt sprays. Furthermore, Bt sprays are non-systemic insecticides and are therefore ineffective against insects that do not come into direct contact with the crystals, such as sap sucking and piercing insects, against root dwelling pests, or larvae that after hatching rapidly burrow or bore into plant tissues. In addition, crops are often subject to predation by a variety of pests that cannot currently be controlled by a single Bt product. The first two problems have been effectively addressed by creating transgenic plants that express the crystal proteins. In these plants, the toxin is continuously produced and protected against degradation, and provided it is expressed in the appropriate tissues it will also be ingested by boring larvae. The problem of narrow specificity may be overcome by simultaneously expressing genes for proteins with different specificities (resistance gene 'stacking' or 'pyramiding'). Transgenic *Bacillus thuringiensis*-plants.

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