Developing Insect-Protected Crop Plants Through the Use of the Insect Control Proteins from *Bacillus thuringiensis*

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Abstract

In 1996 Monsanto commercialized two crops in the United States that were genetically modified to control economically important agricultural pests in potatoes and cotton, NewLeaf™ Potatoes and Bollgard™ Cotton. NewLeaf Potatoes provide season-long control of the Colorado potato beetle, the most damaging insect pest of potatoes in North America. Bollgard cotton provides season-long control of the principal insect pests of cotton, the cotton bollworm and tobacco budworm. On the way, is Yieldgard™ corn , which provides excellent control of the European corn borer and is expected to reach the U.S. marketplace in 1997. These crops have been genetically modified to produce the insect control protein derived from the common soil bacterium *Bacillus thuringiensis (B.t.).* The *B.t.* protein produced by these plants is identical with that found in nature and in commercial *B.t.* formulations, available for the last 30 to 40 years. While present microbe-based *B.t.* products control these same insects, they have shortcomings such as high costs and short half-life in the environment, which have kept them from becoming more widely used. They account for only 1-2% of all the insecticides that are currently used around the world. Through genetic engineering, the gene that specifies the *B.t.* insect control protein is expressed throughout the entire plant, thereby providing continuous protection and greatly expanding the usefulness of this natural pesticide.

Introduction

Today, we live in a world populated by more than 6 billion people. It is projected that this number will double over the next 40 years. This represents a significant population increase, which will also require a tripling in the demand for food. Using current agricultural practices, the destruction of more undeveloped land, is inevitable to satisfy this demand.

The world is already farming approximately six million square miles of land, which represents an area about the size of South America. This is our most productive and sustainable farmland. Without continuing yield increases, the demand for food in the future could require the cultivation of an area equal to North and South America combined. Doing so, would inevitably result in the destruction of wilderness areas and a subsequent loss in valuable biodiversity.

Biotechnology, is obviously not the only answer to solve these problems. However, it can play an important role in meeting the world's increased need for food as we enter the next century. For as stated by Dr. Peter Raven, Director of the Missouri Botanical

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Garden, "The prospects for the intelligent use of genetic engineering are virtually unlimited".

Agricultural biotechnology products have been under development since the early 1980s and the first product, Flavr Savr tomatoes, was commercialized in 1994. Since that time, several more products have reached the marketplace. Agricultural biotechnology is no longer just a research program but a commercial reality. These products include plants with improved agronomic traits such as resistance to insects or viral diseases and tolerance to environmentally compatible herbicides, others have enhanced quality traits and still others will produce pharmaceuticals, chemicals and biofuels.

Today, I will discuss the insect-resistant plants developed by Monsanto, which employ the insect control proteins from *Bacillus thuringiensis*. These products include NewLeaf potatoes, which use the CryIIIA protein from *Bacillus thuringiensis* subsp. *tenebrionis* for control of the Colorado potato beetle and Bollgard cotton and Yieldgard corn which employ the CryIA(c) and CryIA(b) proteins, respectively, from *Bacillus thuringiensis* subsp. *kurstaki* to control the principal lepidopteran pests of these crops. These pests include the cotton bollworm, tobacco and pink bollworm in cotton and the European corn borer and other stalk-boring insects in corn. To maximize the use of the time given to me for this presentation, however, I will focus my comments on NewLeaf potatoes.

NewLeaf potatoes

The Colorado potato beetle, is the most damaging pest of potatoes in North America and parts of Europe. If left untreated the larval and adult stages can completely defoliate the plant resulting in yield losses of more than 85%. In some areas of North America, more than US$200 is spent per acre to control the pest. However, due to insect resistance and environmental factors, the traditional control options have diminished.

In addition to the use of traditional pesticides, farmers have resorted to burning the insects off the plants with propane flamers and even using large vacuum cleaners to remove the pest. Although creative, these methods damage the plants and do not provide superior Colorado potato beetle control.

NewLeaf potatoes, however, provide a number of advantages for Colorado potato beetle control. The *B.t.t.* protein is produced by the plant at a level great enough to provide complete control of both the larvae and adults throughout the growing season. This control results in not only an elimination in the use of insecticides to control this pest, but also a reduction in total grower input costs. In addition, because the *B.t.t.* protein is specific for Colorado potato beetle control, beneficial insects are not affected and are able to increase in number to control other pests of potatoes that are not controlled by this protein, such as aphids and leafhoppers that can transmit viral diseases. This specificity makes NewLeaf potatoes an excellent addition to integrated pest management and biological control programs.

The CryIIIA *B.t.t.* protein produced in NewLeaf potatoes is identical with that found in microbial formulations of *Bacillus thuringiensis* subsp. *tenebrionis* in use since 1988. Although these microbial *B.t.t.* products can provide effective Colorado potato beetle control, they are not widely used because they have a short residual activity in the field, are not effective against larger larvae and adults, and can typically be expensive to use. In the United States they represent approximately 1% of the insecticides used to control this pest.

However, when the *B.t.t.* protein is produced in potatoes, Colorado potato beetle control is outstanding. In field trials conducted in Hermiston, Oregon, NewLeaf potatoes demonstrated their ability to eliminate this pest, the same has also been seen in Antigo, Wisconsin, and in Long Island, New York, where potato production was almost eliminated due to the high degree of resistance to chemical insecticides used to control Colorado potato beetles in this area. These same insects, were, however, very susceptible to the *B.t.t.* protein expressed in NewLeaf potatoes. We have seen similar results throughout the potato growing regions of the United States and Canada.

Also important, is that NewLeaf potatoes look and grow just like any other potatoes. In a business where potatoes are judged by how well they demonstrate particular characteristics of size, shape, composition and texture, NewLeaf potatoes make the grade.

Newleaf Russet Burbank variety potatoes were commercialized in the United States in 1995 and in Canada and the Republic of Georgia in 1996 after the appropriate regulatory approvals were received. Import approval for NewLeaf potato products was received in Mexico in 1996 and in Japan, the Ministry of Health and Welfare is currently reviewing the information necessary to allow the import of NewLeaf potato products into this country as well.

To support these regulatory approvals, the food, feed and environmental safety of NewLeaf potatoes has been addressed. These potatoes represent a valuable tool for Colorado potato beetle control as well as a significant investment. Consequently, we have expended a great deal of effort over the last ten years to develop and implement approaches to prevent or delay the development of resistance to the *B.t.t.* protein produced in these plants.

B.t.t. and NPTII protein safety

The safety assessment for NewLeaf potatoes focused on the safety of the newly expressed proteins and the compositional and nutritional equivalence of these potatoes to commercial Russet Burbank variety potatoes.

As stated earlier, the *B.t.t.* protein produced in NewLeaf potatoes is identical with the CryIIIA protein found in microbial formulations in use since 1988. Because of its receptor-mediated mode of action, the protein is specific for the Colorado potato beetle and safe to non-target organisms such as predaceous and parasitic insects, honeybees, birds, and mammals.

The NPTII protein is used as a selectable marker to promote plant transformation. The protein has no pesticidal activity and is ubiquitous in enteric and soil microbes. The United States Food and Drug Administration approved the use of this protein as a processing aid food additive in several crops and a similar conclusion regarding its safety was made by the World Health Organization.

Monsanto conducted numerous studies to confirm the non-target and environmental safety of the *B.t.t.* and NPTII proteins produced by NewLeaf potatoes. The susceptibility of four orders of beneficial insects and earthworms was evaluated. No adverse effects were demonstrated by any of these organisms. The metabolic fate of the *B.t.t.* and NPTII proteins was also assessed in simulated gastric and intestinal fluids. Both proteins were found to break down in less than 30 seconds in simulated gastric fluid. In simulated intestinal fluid, the *B.t.t.* protein was degraded to a tryptic core, while the NPTII protein was fully degraded in less than five minutes. A mouse acute gavage study was performed at a dose equivalent to over a 2.5 million-fold safety factor based on the average human consumption of potatoes and the level of the *B.t.t.* protein present in the tuber. On the basis of body weights, food consumption and gross Necropsy, no adverse effects were observed.

The environmental fate of the *B.t.t.* protein was also assessed by incubating both NewLeaf potato tissues and purified protein in soil. Complete loss of bioactivity was detected within 9 days. Therefore, a rapid loss of *B.t.t.* protein bioactivity contained within potato plant tissues can be predicted under field conditions.

These studies confirm the safety of these two proteins to nontarget organisms and the environment.

Compositional and nutritional equivalence

The compositional and nutritional equivalence of NewLeaf to commercial Russet Burbank variety potatoes was also assessed. In a series of evaluations, the proximate components: protein, fat, dietary fiber, calories and ash were demonstrated to be equivalent to the levels of these characteristics in non-transformed Russet Burbank potatoes. The same was also found to be true for the principal vitamins produced by potatoes such as: vitamin C, B6, riboflavin and biotin; and the principal minerals such as calcium, copper, potassium and iodine. There was also no difference in the amount of the glycoalkaloid natural toxicants, solanine and chaconine, produced in potatoes. Finally, the wholesomeness of NewLeaf potatoes was evaluated in a 28-day rat feeding study. Again, there was no difference in the amount consumed and weight gained between those rodents fed Newleaf Russet Burbank potato tubers or non-transgenic tubers of the same variety.

Consequently, on the basis of this information, it is clear that NewLeaf potatoes, look the same, grow the same, taste the same and are as wholesome as any other Russet Burbank potatoes. In fact, they are just potatoes but with an ability to resist the feeding of Colorado potato beetles.

Resistance management

Probably the single most important issue we have faced concerning our *B.t.* insect-resistant crops is the potential for insects to develop resistance to the proteins produced by these plants. Monsanto, and other companies producing these products are the greatest stakeholders of this technology because of the years of research, development and investment expended in bringing them to the marketplace. The *B.t.* Management Industry Working Group, was founded in 1988 to enable companies producing either plant or microbial *B.t.* products to address this issue. To that end, the Working Group has provided hundreds of thousands of dollars over the last eight years for resistance management research for academic and government scientists.

As a company, Monsanto was a founder of the *B.t.* Management Industry Working Group and has taken a consistently proactive approach over the last twelve years to research, develop and implement strategies to delay or prevent the development of resistance. This work has been done in collaboration with international pest and resistance management experts from academia, government and industry to develop the most effective strategies for each individual crop. These strategies have been developed, not only for resistance management but also to enable their successful implementation within existing integrated pest management practices.

As a result of this collaboration and extensive laboratory and field research, we are pursuing several resistance management strategies. These include monitoring to assess the susceptibility of our target pests to the *B.t.* protein produced in NewLeaf potatoes, Bollgard cotton and YieldGard corn. Baseline data have been developed and subsequent monitoring will help provide an early warning or confirm whether resistance has developed within a population.

We are also expressing the *B.t.* proteins at a high dose in each crop so as to control both the susceptible insects as well as any of the offspring that may result from a mating of a resistant and susceptible individual. In so doing, any resistant individuals would be very rare and a mating between two of them would be an even rarer event. To encourage the population of susceptible insects, refugia, or non-transgenic plant hosts of these insects will also be maintained. This can be done in several ways, which include the planting of the nontransgenic crop alongside or inter-mixed with the transgenic crop. Alternate hosts of these insect pests, may also be utilized.

It is important to realize that *B.t.* plants are another tool for pest control and therefore, it is important to utilize agronomic practices that are both consistent with sound pest management and are able to minimize exposure of the pests to the crop. These include, using early maturing plant varieties, some method of crop destruction and crop rotations.

Finally, to increase the barriers which a pest would have to overcome to develop resistance, pesticidal traits with different modes of action are being developed. These can include pyramiding the *B.t.* gene with natural host plant resistance traits, such as nectariless or high gossypol cotton or through the insertion of a gene that would produce a protein with a novel mode of action.

However, unless these strategies are implemented, they will not be effective in delaying or preventing resistance development. As stated earlier, industry is the greatest stakeholder of this technology and therefore, has the most to gain in maintaining its durability. Unlike traditional pesticides, the plant is the product. This is an important point for several reasons.

For NewLeaf potatoes, Bollgard cotton and Yieldgard corn, insect resistance is the base product trait upon which other traits such as virus resistance, herbicide tolerance, and quality improvements may be added. Consequently, if resistance develops to the *B.t* protein produced in the plant, not only is the base product lost, but the succeeding products are lost as well. With a traditional pesticide, resistance to a particular pest may mean that the insecticide is not useful in a particular crop, but not necessarily all crops and pests for which that pesticide may be used.

Since the plant is the product, nothing more needs to be applied. Yet for the first time, the target pest and the pesticides applied to control it are greatly reduced, if not completely eliminated. The removal of the primary pest in an agricultural system makes transgenic plants a unique and natural fit within integrated pest management and biological control programs.

Since the plant is the product, we will also have direct contact with each and every grower producing the crop. Consequently, we will be able to provide information on resistance management as well as have a direct mechanism for growers to inform us if they believe resistance is developing. We can then immediately follow-up to confirm and mitigate the problem.

Finally, for the first time, a pesticidal product is being commercialized with a proactive, rather than a reactive resistance management program. Since resistance has not developed we have a much greater opportunity to prevent or delay its occurrence.

Summary

Monsanto has developed several crops with resistance to insects through the use of the insect control proteins of *Bacillus thuringiensis*. Both NewLeaf potatoes and Bollgard cotton have been commercialized in the U.S.and regulatory approvals are being sought in the countries in which these crops will be grown or exported. These products have been demonstrated to be substantially equivalent to their nontransgenic counterparts and are safe to non-target organisms and the environment. Because of the outstanding control they provide for the major insect pests of potatoes, cotton and corn, they represent a valuable and important tool for pest management in these crops. Maintaining their durability is an important concern and proactive resistance management programs are being developed and implemented.