



Pest-Resistance Management Insecticidal Bt-Potatoes



Can you imagine a plant that produces its own insect-killing protein that is highly specific and environmentally safe? Can you imagine that this food-safe protein was originally obtained from a common soil bacterium and not a plant? This is not science fiction but science fact.

In April of 1995 the Environmental Protection Agency (EPA) registered three plant-pesticides that were genetically engineered to produce the protein endotoxin of the soil bacteria *Bacillus thuringiensis* (Bt). Potatoes, corn, cotton, and most recently tomatoes are the first insecticide-producing plants developed with the tools of biotechnology and registered with the EPA under the recent plant-pesticides review. Bt-sweet corn is close to commercial release. In 1997, approximately 40,000 acres of the Bt-endotoxin potatoes were planted for commercial production. The target pest is the Colorado Potato Beetle (adult shown above) which is considered the most devastating insect pest of potatoes. The projection is for between 50,000 to 100,000 acres of Bt-potatoes by 1998. In addition to potatoes, Bt-cotton is expected to exceed 2 million acres and Bt-corn 1 million acres in 1998.

The use of Bt-engineered plants is predicted to significantly reduce the use of synthetic insecticides on these major crops. However, this strategy remains controversial due to largely unresolved concern that broad scale use of Bt-plants will accelerate the development of insensitivity to control among the target insect pests.

Science Provides Evidence for Rates of Pest Resistance Recent risk assessment reports have effectively demonstrated the potential for faster adaptation of insect pests to Bt-toxins. A greater number of insects in the populations studied were found to have a genetic resistance for Bt. With continual exposure to high levels of protein toxin found in Bt-plants, the resistant forms are most likely to survive and interbreed. Interbreeding raises the resistance of the pest to the current forms of Bt and by cross resistance, possibly to other forms of Bt not yet released. The pest tobacco budworm (*Heliothis virescens*) was found to have genetic resistance in 1 in 350 individuals collected in agricultural production fields (cotton). These findings demonstrate pest resistance potential far higher than predicted by earlier theoretical models. Experts are predicting a 3-4 year durability cycle for highly planted Bt-crops, even with current resistance management procedures.

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Pest Resistance Management

Strategies to achieve biotechnology-based plant tolerance or resistance to herbicides, insect pests, and pathogens such as viruses, bacteria, fungi, nematodes, and parasitic plants have raised concern due to the high potential to result in pest-insensitivity. This acquired insensitivity, or Pest Resistance, is well documented for both chemical pesticides and plant resistance traits that originate from a single, dominant genetic element. New strategies are being developed to maintain a pest population sensitive to the control practice. This is an essential element of the implementation of these new technologies and products to preserve their effectiveness over many seasons.

For Bt-producing crops, the key Pest Resistance Management strategies involve;

- ◆ High protein-toxin production in the plant to kill all but the rare, highly insensitive pests.
- ◆ Bt-free crop sanctuaries, termed refugia, to maintain a Bt-sensitive breeding population of the pest. This population will exceed the number of insensitive individuals and, through mating, will effectively prevent a highly resistant population from developing.

Before discussing the merits of this management scheme in more detail it is important to review some basic facts about Bt-insecticides.

What is Bt-toxin?

The Bt-toxin is a familiar pest management product to commercial growers and home gardeners, primarily for the control of lepidopterous pests such as cabbage loopers and tomato hornworm (See Technology Focus on page 6). Many forms of Bt-toxin have been registered with the EPA to control many types of insect pests. These recent products of biotechnology-based breeding, mentioned above, were genetically-modified to produce the familiar *Bt* insecticidal crystal-protein, normally applied to plants as a granular dust, during otherwise normal plant growth. *Bt*'s have been used effectively in vegetable production, including organic farming, for over 40 years. Bt-based insecticides are well known for their environmental safety and absence of mammalian toxicity. Activation of the toxin requires the alkaline conditions found in insect guts rather than the acidic conditions of mammalian gastric fluids. In addition, the Bt-toxin breaks down rapidly in the environment to non-toxic products. This rapid inactivation by UV light, for example, has limited the stability and effectiveness of spray-on Bt applications. Achieving stability of Bt-toxin by production inside the plant cells is one of the demonstrated benefits of these new plant cultivars.

Russet Burbank, Atlantic and Superior cultivars of potatoes are now commercially available as Newleaf™ lines from NatureMark, a division of Monsanto Corp., that produce their own Bt-toxin protein. These pesticide producing NewLeaf™ cultivars are generally regarded as safe, highly effective and environmentally compatible. The long-term effectiveness of genetically engineered plants to protect against devastating pests like the Colorado Potato Beetle will depend on careful and vigilant **Pest Resistance Management**.

The potential for rapid development of insensitivity to pest control sprays or pesticide-producing plants, is widely recognized for a large family of *Bt* proteins, herbicides, virus resistance and other chemical and genetic pest management strategies. Experts agree that more information on resistance management strategies is essential to protect the useful life of newly registered Bt-toxin producing potatoes as well as the traditional spray-on *Bt* formulations. By preventing the build-up of insect

pests resistant to the plant-produced protein, the effectiveness of control will be sustained for many years.

Why have Bt potatoes raised concern?

The genetic tools of biotechnology are being used to introduce single pest control or chemical tolerance (such as herbicides) traits to plants. This is being done with the hope of reducing the use of synthetic pesticide sprays. Alternatively, the anticipated benefit would be to substitute more environmentally benign or ultra-low dose materials as replacements for the more objectionable compounds.

Concern has been raised, however, that these “single-gene” strategies, already in commercial plantings, will cause more pests to become insensitive to both plant-based pest resistance and existing environmentally-friendly agricultural sprays, like the *Bt* protein. Resistance can arise relatively rapidly because, often, a proportion of pests in any field population are naturally insensitive to some chemical and plant-based, genetic control factors. Also, resistance to one material may give the pest tolerance to related compounds, called cross-resistance.

These insensitive forms of the pest have an advantage in survival, growth and reproduction with repeated use of the pesticide or genetically resistant plant cultivar. The insensitive population increases as the resistance trait (resistant to the chemical or plant resistance factor) increases to become the greater population through more frequent breeding encounters. Bt-insensitive insects selected by the misuse of plant-based Bt's, it is argued, will eliminate this valued pest management tool for both technology users and non-users alike. For more background on selected insensitivity to the Bt protein; see the Technology Focus on page 6.

What is Pest Resistance Management?

Repeated applications of a single pesticide (or plant-based pest resistance trait) have the potential to increase the abundance of pests that are insensitive to existing or recently introduced spray-products or pest resistant vegetable cultivars. The problem of pesticide resistance in conventional agriculture is already a serious one, estimated to contribute to as much as 25% of the annual pest control expenditures in the United States.

Though contrary to the notion of killing agricultural pests, as discussed above, complex schemes are being developed to provide plant sanctuaries, termed refugia, to maintain populations of pesticide-sensitive crop pests. The challenge and expectation for the biotechnology industry will be to evolve from a market-driven approach to eliminate target pests to one where profitability is sustained by integrating products into a managed agroecosystem. Refugia and alternating seasonal use of genetically-modified crops are two components of this strategy.

In the absence of a comprehensive Resistance Management Plan, it is increasingly accepted that widespread *Bt*-resistance (insensitivity of insect pests to the *Bt* protein-toxin) would develop in less than 5 years. Uniform cropping over large acreage's would be a required factor for the population of insensitive forms of the pest to increase at this predicted rate. The best methods to prevent selection for insensitive pest populations remain controversial and have limited supporting field validation data. As with any new technology, that does not have the benefit of use over long periods of time caution seems well founded. A regional approach will likely be required to most effectively manage pest resistance. This debate is on-going in open scientific and public forums. Participation in this debate by environmental, agricultural resource preservation and public advocacy groups has had a

strong influence on the commercial development, registration and marketing of the new *Bt*- protein producing crop plants and their resistance management programs.

The bottom-line is that the management of the rate at which pests become resistant to chemical control agents, biological controls, genetic resistance from traditional plant breeding, or pest resistance achieved by molecular genetic modification has become a critical agricultural issue.

What are the key concerns in Pest Resistance Management?

- ◆ Concerns are greatest and most immediate for biotechnology-modified plants that are being marketed with insecticidal, virus resistance, or herbicide-tolerance traits. The key issues are ;
- ◆ Cross-resistance; Resistance to one pesticidal product confers resistance to related chemistries
- ◆ Cross-breeding of introduced resistance factors to native plants, especially in centers of crop origin or genetic diversity
- ◆ Potential for increased use of broad-spectrum chemical pesticides that are co-marketed with biotechnology -developed crops
- ◆ Acquisition of novel traits by pathogens that break plant genetic-resistance or give novel virulence (aggressiveness) or host range

For *Bt*-potatoes, the potential of cross-breeding to native plants is considered to be extremely unlikely. Two species of non-tuber forming relatives of the domestic potato, *Solanum tuberosum* L., are found in the United States. These are *Solanum fendleri* and *Solanum jamesii*. Geographic isolation of these related species from potato production fields and further isolation due to genetic cross-breeding barriers were among the criteria used by the EPA to allow registration of the *Bt*-protein producing plants.

Who regulates Bt-potatoes and enforces Pest Resistance Management?

Commercializing companies, academic research leaders, environmental organizations, and state and federal regulatory agencies are working together to develop the best management strategies for preserving the effectiveness of existing and newly developed pest control products. In response, in part, to the rapid commercial development of insect -resistant crops by molecular breeding (genetic engineering), the Environmental Protection Agency (EPA) formed the Pesticide Resistance Management Workgroup (PRMW) in 1988. The advisory board of experts have representatives from plant pathology, microbiology, entomology, weed science, systems-ecology, and biochemistry. This board has been developing the science-based guidelines and criteria for establishing resistance management plans that must include;

1. Knowledge of the pest biology and ecology
2. Effective strategy for preserving genetic resistance resources
3. Effective refugia recommendations (Refugia are essentially pesticide-free crop sanctuaries for the sensitive pest population)
4. Resistance development monitoring, reporting and follow-up mitigation plan
5. Incorporation of Integrated Pest Management (IPM) strategies into technical cropping recommendations
6. Communication and education programs for responsible product use

7. Commitment to develop alternate chemistries or activities and genetic strategies that will delay or prevent pest resistance build-up

These crop and pest-based strategies represent the best currently known and practical approaches to preserve the longevity of Bt-based pesticides for everyone. The specifics of some elements of the plan remain largely unanswerable until experience-in-use is available. These include;

- ◆ Design and management of refugia. (The optimal pattern, ratio to Bt-crop, and especially distances of refugia from main Bt-crop are not well understood. Expert opinion suggests that a minimum of 25% non-Bt plants in a patchwork pattern should be used but in current practice as little as 4% is committed to refugia.)
- ◆ Pesticide resistance-gene selection rates among pests in field conditions
- ◆ Out-crossing to related inter-breeding native species
- ◆ Variability in Bt-protein production levels under field stress conditions (High dose strategies, to kill all but the most resistant individuals, are believed to be required for effective pest resistance management. Bt-potatoes are engineered for high dose delivery. Field and environment induced variability may result in lower dose delivery than expected. This could accelerate the development of insensitivity among insect populations)

Have food safety concerns been addressed?

Over 176 different Bt products have been registered for commercial use since 1961. No dietary toxicity to vertebrate species (mammals, fish, birds) has ever been reported. Digestion studies have shown that the *Bt*-protein is degraded in mammalian gastric fluids (digestive acids and enzymes) in less than 30 seconds. No allergenic reactions are known for *Bt*-proteins. The *Bt*-proteins do not have the common traits of an allergen. Unlike the majority of known allergens, they are inactivated by heat, acids and protein degrading enzymes. They are also not specially modified by the plant (called glycosylated) and are present in relatively low concentrations. Bt-proteins, whether as spray-on formulations or produced by plants, are considered very food-safe.

What is the impact on vegetable production in California?

For vegetable production in California, *Bt*-protein cultivars are not on the immediate commercial horizon. NewLeaf™ potatoes from NatureMark (a Monsanto Corp. subsidiary), modified to produce the *Bt*-protein-toxin, will be grown in other states and reach consumers in 1996-97. Cotton producing the *Bt*-protein cotton will be marketed for planting in California in 1997.

Biotechnology-based virus resistance in yellow crook-neck squash (FreedomII from Asgrow Seed Co.), other squash types, melons, watermelons, tomatoes and peppers is being evaluated in California fields. The number of virus-resistant cultivars and multiple resistance lines in the EPA registration process is growing rapidly.

Looking forward, for *Bt*-insecticides, herbicide-tolerance traits, virus resistance factors or any other pest control strategy, pest resistance management will be an increasingly scrutinized component of pesticide and plant-pesticide review, registration and marketing.

Biotech Potatoes in the Pipeline

NewLeaf™ Predicted release date	Bt-protein Insect resistance	Potato Leafroll Virus Virus resistance	Potato Virus Y Virus resistance	Verticillium Fungal Wilt resistance	Late Blight resistance	Improved Solids Bruise resistance
Russet Burbank	1995	1998	1999	2000+	2000+	2002+
Atlantic	1997					
Superior	1997					
Russet Norkotah	2000	2000				
Shepody	2000	2000				
NewLeaf Ultra	2000+	2000+	2000+	2000+	2000+	2002+

Other projected bioengineered traits include bacterial soft rot resistance and glyphosate herbicide resistance (Roundup Ready®).

Technology Focus: Bioengineering a Natural Insecticide

Bacillus thuringiensis (Bt) is a common soil bacterium that produces an insecticidal protein crystal, during dormancy, as part of its natural life cycle. Applied to crops and home garden plants as a spray or granular dust, the crystal, or *Bt*-protein, is ingested during insect feeding. The insect gut activates the protein into a toxic form. This insect killing property has been utilized in vegetable and other plant protection systems for over 40 years. There are many forms of these *Bt*-proteins, each having a defined specificity or narrow range of insecticidal abilities.

With modern biotechnology, the genes for making *Bt*-protein are readily isolated and introduced into plants and become a stable inherited component of a new cultivar. Over 50 distinct *Bt*- proteins have been characterized with inhibitory effects on pest moths, beetles, flies, mosquitoes, and other major insects. A change in a single amino acid in the *Bt*-protein has been shown to have dramatic effects on the range and specificity of activity against an insect pest species.

This specificity contributes to the potential for rapid development of resistance. To date, while only two insect species have been shown to have become insensitive to a specific *Bt*- protein in the field, more than ten species have developed resistance in laboratory studies.

More alarming was the discovery that with one important pest, tobacco budworm, insensitivity to one *Bt*-protein also conferred high levels of tolerance to several other *Bt* crystal toxins. Cross-resistance such as this is a critical issue in the debate over the large-scale cropping of *Bt*-protein producing plants.

Using a detailed knowledge of the specific essential amino acid arrangements, commercial developers are racing to design more effective and more specific “ smart *Bt*-proteins” that combine elements of natural genetic-specificity determinants to customize the range of activity and mode of

action of novel *Bt*-proteins. This protein engineering is predicted to introduce new activities against insect pests not known to have a natural sensitivity to *Bt*-proteins. This approach provokes both praise for providing environmentally-compatible solutions and predictions of ecosystem disruption. Altering specificity to insect pests and modifying the insecticidal target-site in the insect are viewed by many researchers as providing, in part, the dynamic strategy essential for pest resistance management.

Another suggested strategy may be the use of “helper toxins”, also produced by some *B. thuringiensis* subspecies. Recent studies by entomologists at UC Riverside have shown that sublethal doses of a second protein toxin, CytA, overcomes high levels of insensitivity to Bt-protein in mosquito larvae. CytA has a different cellular target site than Bt-protein. Other natural or synthetically designed co-toxins may be found for Colorado Potato Beetle and other vegetable pests.

For More Information On Pest-Resistance Management, Bt-products, or Agricultural Biotechnology:

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Photo Credit: Adult Colorado Potato Beetle - Clemson University Dept. of Entomology,
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