

**BOLLGARD COTTON:  
RECENT DEVELOPMENTS  
FROM LAB TO MARKETPLACE**  
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**Abstract**

The development of insect-protected cotton through the use of transgenic technology has progressed rapidly in recent years as illustrated by the success of Monsanto's Bollgard cotton, now available in the marketplace in a number of commercial backgrounds. The research and development process needed to successfully develop additional transgenic cotton insect control products is outlined and illustrated with examples of potential future products at various stages within the process. This brief review will focus on several technical aspects of success.

**Introduction**

Within Monsanto's program for the development of insect-protected cotton there are several levels of research. In order for new, valuable traits to be introduced and successfully utilized in the marketplace, several obvious technical hurdles must be cleared:

- 1) Discovery & Development- New genes for the control of agronomically important cotton pests must be discovered and fully characterized, both for efficacy (value) and safety.
- 2) Transformation - Appropriate genes must be incorporated into the cotton genome.
- 3) Laboratory and field evaluation - The new gene-plant background combination must be fully evaluated for efficacy and agronomics.

**Discussion**

Within Monsanto's insecticidal gene discovery program, tens of thousands of protein samples have been screened against a number of cotton pests over the last 10 years. The largest source of protein mixtures for testing has come from the fermentation of microbes. Both Bt and non-Bt microbial isolates have been tested in the screening process. Insecticidal proteins discovered include numerous "Cry" proteins from a variety of Bt isolates and a few non-Bt proteins, including cholesterol oxidase, which was isolated from a *Streptomyces* isolate and is active against the boll

weevil. In addition, plant protein extracts and commercially available enzymes have served as sources of potential insecticidal proteins.

The richness and variety of insect-active proteins found among the numerous isolates of *Bacillus thuringiensis* have allowed for the "engineering" of unique toxins. Portions of the Bollgard gene (*cryIAc*) and the *cryIF* genes have been combined to form a toxin that retains the characteristic CryIAc activity against Bollgard's main targets (*Helicoverpa zea*, *Heliothis virescens*, and *Pectinophora gossypiella*) while adding significant activity against several *Spodoptera* species including beet armyworm and fall armyworm.

Once candidate toxin genes have been identified, fully characterized, and purified, the specific genes encoding those proteins are isolated. Cotton plants are then transformed through the introduction of the genes into undifferentiated cotton cells (callus) which are subsequently cloned to form individual plants which are then propagated. In many cases, molecular biologists are able to optimize these toxin genes for expression in plants by altering some DNA bases and thereby rendering the gene more "plant-like" without changing the primary structure of the resulting protein. Genes are introduced either through an engineered version of the gall-forming bacterium (*Agrobacterium* species), or via "gene gun". The altered agrobacterium inserts the insect toxin gene (instead of its normal gall-forming genes) into the chromosomes of the plant cells; the gene gun forces strands of engineered DNA (annealed to small bits of gold) into the nuclei of plant cells where the DNA becomes incorporated into the chromosomal plant DNA. The resultant cloned plants must be tested for efficacy against insects and for agronomic abnormalities.

Through this process, Monsanto is attempting to provide a continuous supply of genes to be evaluated *in-planta* for their potential to provide resistance management utility and expand Bollgard's target pest spectrum. During 1999 Monsanto will be evaluating a new gene as an addition to Bollgard in a stacked-gene product. This second gene, labeled *cryX*, encodes a lepidopteran-active protein with potential IRM value. Anticipated 1999 studies include 50+ acres of field-testing for insect efficacy and agronomic performance. It is our hope that at next year's Beltwide meeting we may provide significant information on the potential value of *cryX*.