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<u>Abstract</u>

A new registered insecticide named CRYMAX contains *Bacillus thuringiensis* strain EG7841. EG7841 was developed utilizing several techniques to contain an optimum combination of insecticidal proteins for control of a variety of lepidopterous cotton pests.

Introduction

Insecticides based on the soil bacterium *Bacillus thuringiensis* have become a vital part of insect pest management in much of the cotton growing region of the United States. The primary role of these products has been for the control of the tobacco budworm/bollworm *(Heliothis/Helicoverpa)* complex. During the past decade, the level of active ingredient and the potency of the active ingredient have been the primary areas of improvement. Recently developed techniques in recombinant DNA have opened new possibilities for improving BT-based insecticides. Such techniques were utilized in the construction of BT strain EG7841.

Discussion

Bacillus thuringiensis produces a variety of insecticidal proteins that are toxic to a number of insect species including many lepidoptera that cause economic crop damage. During the 1970's and early 1980's, BT research focused on isolating naturally occurring BT strains with improved insecticidal activity and improving the manufacturing and formulation of products based on the HD-1 isolate. As the 1980's progressed, it became evident that different specific proteins had widely different activities against different insect species. In cotton production the development of pyrethroid resistance in tobacco budworm, which originated in east Texas and has spread eastward. led to increased use of BT insecticides as a tool in managing resistance. One of the insecticides that has gained widespread use in cotton is Condor bioinsecticide. Condor features the BT strain EG2348 as an active ingredient and was one of the first strains constructed using conjugal transfer to improve the insecticidal activity against the Heliothis/Helicoverpa complex.. With the advent of a more potent product, the use of Condor expanded into various season-long strategies in addition to management of pyrethroid resistance.

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In order to develop an improved product that could eventually replace or complement Condor for cotton insect control, Ecogen scientists focused on improving the insecticidal activity against the budworm/bollworm complex as well as secondary pests such as beet armyworm and soybean and cabbage loopers. In order to determine the desired direction for strain development, a technology had to be developed that allowed the cloning of single BT genes for expression in a previously crystal-free BT strain. This technique allowed for the comparison of individual insecticidal proteins that had been expressed in a BT strain. After determining which genes were desirable, another technology had to be developed which allowed for their expression in a BT strain without the use of foreign DNA. This technique has been named the SSR, or Site-Specific Recombination system.

By comparing a variety of different single proteins, it was determined that genes of the *cry*1Ac, *cry*2A and *cry*1C classes would provide an optimal combination of insecticidal proteins. The Cry1Ac and Cry2A proteins provide excellent activity against the budworm/bollworm complex and loopers. A Cry1C protein provides activity against beet armyworm and loopers. Using new strain discovery, conjugal transfer, and the SSR system, BT strain EG7841 was constructed that contains three *cry*1Ac genes, and single *cry*2A and *cry*1C genes. Just as noteworthy, BT strain EG7841 does not contain any less desirable genes which produce proteins that dilute the more active proteins.

CRYMAX contains the BT strain EG7841 as the active ingredient and has been formulated as a 15% water dispersible granule and a 10% oil flowable. Compared to Condor XL which contains 15% active ingredient, either CRYMAX formulation has significantly better activity in laboratory bioassay against tobacco budworm, cotton bollworm, beet armyworm, loopers, and European corn borer. CRYMAX WDG gained EPA registration during 1996 and has been introduced in vegetable markets. Field tests during 1996 in cotton and soybeans focused on evaluating the oil flowable formulation. Results indicated that CRYMAX performed equal to or better than Condor XL at equivalent product use rates against the budworm/bollworm complex and loopers. Populations of beet armyworm were light during 1996 and meaningful tests in cotton were not accomplished although evaluations of CRYMAX WDG for control of beet armyworm in vegetables have been highly successful. Plans for evaluating CRYMAX in 1997 include large-scale whole field evaluations was well as additional small-plot testing.

Summary

A new BT strain, designated EG7841, has been constructed utilizing multiple genetic techniques to combine various insecticidal proteins for optimal activity against tobacco budworm, cotton bollworm, beet armyworm, cabbage and soybean loopers. CRYMAX, a product containing EG7841, has demonstrated significantly improved activity in laboratory bioassays compared to Condor XL against these cotton insect pests. Initial field evaluations conducted in 1996 have supported the laboratory data and such tests will be expanded in 1997.