

**BOLLGARD®II: IMPROVEMENTS IN EFFICACY AND SPECTRUM AGAINST LEPIDOPTERAN
PESTS OF COTTON**

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Abstract

A technological milestone in genetic engineering resulted in the production and deployment of Bollgard® cotton varieties expressing the Cry1Ac -endotoxin, for the control of key lepidopterous pests of cotton. Commercially available in several varieties since 1996, this product has provided an effective and specific alternative to the use of chemical insecticides for the control of bollworm, *Helicoverpa zea* (Boddie); pink bollworm, *Pectinophora gossypiella* (Saunders) and tobacco budworm, *Heliothis virescens* (F.). In an attempt to increase efficacy, expand spectrum of activity, and mitigate or postpone the development of resistance, a stacked product, Bollgard II®, was developed, expressing both Cry1Ac and Cry2Ab2 proteins. The combined activity of the two proteins in Bollgard II provides increased efficacy against the budworm/bollworm complex, and enhanced spectrum of activity against beet armyworm, *Spodoptera exigua* Hübner; fall armyworm, *Spodoptera frugiperda* (J. E. Smith) and many other sporadic lepidopteran pests of cotton.

Studies were conducted on fresh and lyophilized conventional, Bollgard and Bollgard II tissue from greenhouse grown plants and using whole plants in growth chamber experiments. The expression profile and bioactivity of different parts of the cotton flower relative to vegetative parts was assessed. Large leaves, terminal leaves, squares, flowers and small bolls (under bloom tags) were sampled from three near-isolines, DP50, DP50 Bollgard® and DP50 Bollgard II®. The flowers were subdivided into the component parts - bracts, calyx, petals, anthers, and ovules. Most Bollgard II tissues gave significantly higher mortality relative to DP50 tissues. Larvae surviving on Bollgard II were severely stunted, demonstrating the high level of bollworm control that it provides compared to Bollgard.

Expression profile studies on Bollgard and Bollgard II plants using the tobacco budworm quantitative bioassay and ELISA showed that all flower tissues under investigation expressed the Cry1Ac alone or in combination with the Cry2Ab2 proteins, and in many instances, at levels comparable with expression in terminal leaf or square tissues. Significantly higher lepidopteran activity was demonstrated when using Bollgard II tissues, relative to Bollgard, across all tissue types, demonstrating the increased levels of insect control provided by the Cry2Ab2 gene in Bollgard II. The *in vivo* growth chamber experiment demonstrated clearly that when flowers were infested with newly emerged neonates, 24, and 48 h diet-fed *H. zea* larvae, Bollgard II provided a higher level of fruit protection than Bollgard as measured by the abscission of the floral structures.