2003 BELTWIDE COTTON CONFERENCES, NASHVILLE, TN – JANUARY 6-10

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

S. Sivasupramaniam, L.G. Ruschke, J.A. Osborn, M.E. Oppenhuizen, J.W. Mullins, K.G. Watson, and G. Mueller
Monsanto Company
St. Louis, MO

Abstract

A technological milestone in genetic engineering resulted in the production and deployment of Bollgard® cotton varieties expressing the Cry1Ac protein, for the control of certain lepidopterous pests of cotton. Commercially available in several varieties since 1996, this product has provided an effective and specific alternative to the use of synthetic insecticides for the control of tobacco budworm, *Heliothis virescens*; cotton bollworm, *Helicoverpa zea*; and pink bollworm, *Pectinophora gossypiella*. In an attempt to increase efficacy, expand spectrum of activity, and mitigate or postpone the development of resistance, a stacked product, Bollgard II™, has been 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, enhanced spectrum of activity against beet armyworm, *Spodoptera exigua*;; and fall armyworm, *Spodoptera frugi[perda* (Table 1). The Environmental Protection Agency recently granted registration for this product, and Bollgard II™ will be launched in the 2003 cotton-growing season.

Bollgard® cotton has consistently provided outstanding control of the tobacco budworm, *Heliothis virescens*; and pink bollworm, *Pectinophora gossypiella* across the cotton belt, in a diverse number of cotton varieties (Perlak et al., 2001). Although the Cry1Ac protein has very good activity against all budworm/bollworm complex of pests, it has been well demonstrated that especially under high infestations, bollworm larvae have been found feeding in fresh flowers and on small bolls under the bloom tags of Bollgard® cotton (Brickle et al., 2001) and more recently in Bollgard II™ cotton (Gore et al., 2001).

The study was designed to evaluate the expression profile and bioactivity of different parts of the cotton flower relative to vegetative parts. Large leaves, terminal leaves, squares, flowers and small bolls (under bloom tags) were sampled from three isolines, DP50, DP50B (Bollgard®) and DP50B II (Bollgard II™), and the flowers were teased out into the bracts, calyx, petals, anthers, and ovules. These tissue types were freeze-dried, finely powdered and utilized in all assays. The ELISA and the tobacco budworm quantitative bioassay (Greenplate, 1999) were conducted to study the expression profile. Activity against bollworm larvae was ascertained using a diet-based assay, where the tissue in agar was overlaid on diet (2% tissue in 0.2% agar), and infested with first instar larvae. Readings were taken seven days after infestation.

All data (weight data, quantitative ELISA and the tobacco budworm quantitative bioassay data) were subjected to a two-way analysis of variance using PROC GLM (SAS, Version 8), and means for each treatment were separated ($P<0.05$) using Fisher's Protected Least Significance. Bollworm bioassay results were analyzed using the mean weights of surviving larvae (Table 2). All Bollgard® and Bollgard II™ tissues gave significantly lower weight of survivors compared to DP50. Mean weight of surviving larvae fed on Bollgard II™ were significantly lower in weight relative to those fed on Bollgard®, for all tissues. None of the Bollgard II™ survivors developed past the second larval stage, seven days post infestation, demonstrating that it would provide a high degree of bollworm control.

Results obtained using ELISA (Table 3) and quantitative bioassays (Table 4) show that all 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, which are customarily sampled. The results from the quantitative bioassay showed that both Bollgard® and Bollgard II™ did not show significant differences in expression profile among the nine tissue types that were studied. Significantly higher lepidopteran activity was demonstrated by Bollgard II™ relative to Bollgard®, across all tissue types, clearly demonstrating the added value provided by the Cry2Ab2 gene in Bollgard II™.

These studies have clearly demonstrated that most of the floral tissue types examined express the relevant proteins at levels comparable to those found in leaf tissues. It is well understood that Bollgard® provides good but not complete control of the bollworm. Field observations on surviving bollworm larvae in blooms and bloom tags could at least in part be the result of the preferential feeding of bollworm larvae on these plant parts. These and all other studies in our laboratories have shown that mortality measurements alone do not provide the whole picture when considering *in planta* control provided by *Bacillus thuringiensis* toxins. Damage to plant parts and developmental data on surviving larvae should be taken into account before arriving at conclusions with regard to spray thresholds or economic injury. Clearly, Bollgard II™ would provide significantly superior control of bollworms and also has added value from the standpoint of resistance management.

Literature Cited

Brickle, D. S., S. G. Turnipseed and M. J. Sullivan. 2001. Efficacy of insecticides of different chemistries against *Helicoverpa zea* (Lepidoptera: Noctuiidae) in transgenic *Bacillus thuringiensis* and conventional cotton. J. Econ. Entomol. 94: 86-92.

Greenplate, J. T. 1999. Quantification of *Bacillus thuringiensis* insect control protein Cry1Ac over time in Bollgard® cotton fruit and terminals. J. Econ. Entomol. 92: 1377-1383.

Gore, J., B. R. Leonard and J. J. Adamcyzk. 2001. Bollworm (Lepidoptera: Noctuiidae) survival on 'Bollgard®' and 'Bollgard II' cotton flower bud and flower components. J. Econ. Entomol. 94: 1445-1451.

Perlak, F. J., R. W. Deaton, T. A. Armstrong, R. L. Fuchs, S. R. Sims, J. T. Greenplate and D. A. Fischhoff. 1990: Insect resistant cotton plants. Bio/Technology. 8: 939-943.

Perlak, F. J.; M. Oppenhuizen, K. Gustafson, R. Voth, S. Sivasupramaniam, D. Heering, B. Carey, R. A. Ihrig and J. K. Roberts. 2001. Development and commercial use of Bollgard® cotton in the USA – early promises versus today's reality. Plant J. 27: 489-501.

SAS Institute. Version 8, 1999-2001. Cary, NC.

Table 1. Toxicity data (LC_{50}, ug/ml) on Cry1Ac and Cry2Ab2 against important lepidopteran pests of cotton.

Insect	Cry1Ac	Cry2Ab2
Tobacco budworm	0.02	0.44
Cotton bollworm	2.11	16.75
Pink bollworm	0.01	0.04
Beet armyworm	>>100	43.81
Fall armyworm	>>100	76.31

Table 2. Estimated mean weights of surviving bollworm larvae in diet-overlay bioassay using conventional (DP50), Bollgard, and Bollgard II cotton isolines. Lyophilized tissue (2% in 0.2% agar) was overlaid on 500ul of diet in 128-well CDI trays, and neonate bollworms were infested with a fine paint brush at the rate of one insect per well (n=16, per treatment).

	DP50			Bollgard			Bollgard II		
Plant Part	Mean	Sig.	SEM	Mean	Sig.	SEM	Mean	Sig.	SEM
Large Leaf	77.3	a *#	7.2	27.8	a *	3.8	6.1	b	1.5
Terminal Leaf	63.5	ab *#	6.3	19.0	bc *	3.5	5.7	b	0.8
Square	48.8	b *#	5.5	12.6	cde *	2.0	3.4	b	0.4
Bract	80.0	a *#	4.7	28.8	a *	3.1	10.5	a	1.1
Calyx	83.8	a *#	5.1	25.5	ab *	3.8	12.5	a	1.4
Petal	56.2	ab *#	4.2	16.7	bcd *	2.6	5.4	b	0.9
Anthers	39.3	c *#	6.9	14.7	cd *	1.9	5.6	b	0.7
Ovule	25.0	d *#	3.4	8.9	e *	1.8	3.2	b	0.4
Small Boll	26.5	d *#	5.1	11.1	de *	1.5	3.6	b	0.7

* within rows,indicates a significant difference between Bollgard II and Bollgard or DP50 ($p<0.05$)
# within rows, indicates a significant difference between Bollgard and DP50 ($p<0.05$)
Floral part means within columns labeled with the same letter are not significantly different ($P>0.05$)

Table 3. Estimated mean Cry1Ac (in Bollgard, and Bollgard II) and Cry2Ab2 (in Bollgard II) expression profile in different floral tissues using ELISA. Units in µg/g dry weight.

	Cry1Ac, in Bollgard			Cry2Ab2, in Bollgard and Bollgard II		
Plant Part	Mean	Sig.	SEM	Mean	Sig.	SEM
Large Leaf	0.92	b	0.44	419	ab	70
Terminal Leaf	8.33	a	3.42	372	ab	162
Square	4.79	a	0.47	642	ab	86
Bract	0.62	b	0.22	302	b	145
Calyx	1.26	b	0.18	137	b	16
Petal	5.57	a	0.59	380	b	40
Anthers	5.84	a	0.35	583	ab	27
Ovule	4.53	a	1.14	1243	a	294
Small Boll	4.98	a	0.55	792	ab	114

Floral part means within columns labeled with the same letter are not significantly different ($P>0.05$)

Table 4. Estimated mean expression profile in different floral tissues using the tobacco budworm quantitative bioassay. Units in µg/g dry weight, Cry1Ac equivalents.

	Bollgard			Bollgard II		
Plant Part	Mean	Sig.	SEM	Mean	Sig.	SEM
Large Leaf	21.23	a *	.	200	a	.
Terminal Leaf	36.06	a *	.	263	a	.
Square	21.60	a *	9.09	221	a	62
Bract	10.36	a *	7.73	110	a	10
Calyx	8.85	a *	4.19	43	a	10
Petal	34.46	a *	15.92	90	a	5
Anthers	24.46	a *	21.27	68	a	12
Ovule	22.31	a *	13.51	170	a	45
Small Boll	22.64	a *	9.32	198	a	69

* within rows,indicates a significant difference between Bollgard II and Bollgard or DP50 ($p<0.05$)
# within rows, indicates a significant difference between Bollgard and DP50 ($p<0.05$)
Floral part means within columns labeled with the same letter are not significantly different ($P>0.05$)