UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PREVENTION,
PESTICIDES AND TOXIC
SUBSTANCES

April 22, 2004

MEMORANDUM

SUBJECT: Technical review of Monsanto's submission: "Final Report on Studies to Assess Supplemental Pyrethroid Spray Effects on *Helicoverpa zea* Populations in Bollgard®[1] Cotton"
EPA Reg. No. 524-478; Submission dated March 13, 2004 (MRID 462224-02)

TO: Leonard Cole (PM-90)
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PEER REVIEW: Alan H. Reynolds, M.S., Entomologist
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ACTION REQUESTED: Provide a technical review of Monsanto's submission: "Final Report on Studies to Assess Supplemental Pyrethroid Spray Effects on *Helicoverpa zea* Populations in Bollgard® Cotton" submitted as part of the terms and conditions

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of EPA Reg. No. 524-478 (September 29, 2001).

CONCLUSIONS

1. Pyrethroid oversprays in Bollgard cotton fields will increase the level of control of CBW, delay the evolution of resistance (see Gustafson et al.'s (2004) model predictions), and support the continuation of the 5% external, unsprayed, structured refuge requirement. The vast majority of field studies conducted in North Carolina, Louisiana, Mississippi, and South Carolina indicate that pyrethroid oversprays caused a greater percent reduction in cotton bollworm (CBW) infestation levels or boll/square/plant damage in Bollgard cotton fields than in non-Bollgard cotton fields whether irrigated or non-irrigated. These four states represent a range of cotton production conditions across the Cotton Belt. Results should be used to revised the parameter estimations in Gustafson et al.'s (2004) model for mathematical prediction of CBW resistance evolution.

2. Results from North Carolina and Mississippi field studies suggest that pyrethroid oversprays will likely not be necessary for cotton bollworm control in Bollgard II cotton fields as they were for Bollgard cotton fields. Pyrethroid sprays on Bollgard II plots do not provide a statistically significant difference in reduction of CBW infestation or damage from untreated Bollgard II cotton fields or from treated Bollgard cotton fields. Thus, pyrethroid oversprays, as a parameter in the Gustafson et al. (2004) resistance model should not significantly impact the model output for CBW on Bollgard II cotton. Pyrethroid oversprays are not an important parameter in the Gustafson et al. (2004) model for CBW resistance management to Bollgard II cotton.

3. Cotton bollworm (CBW), *Helicoverpa zea*, larvae (late instar larve (L4-L5)) in North Carolina Bollgard and non-Bollgard plots in North Carolina were half as susceptible to Cry1Ac than were populations generated from non-Bollgard cotton survivors in the F1 generation, but this difference disappeared in the F2 generation. There was no statistical difference between these two groups in terms of their susceptibility to cypermethrin at either the F1 or F2 generations.

4. Results from these CBW studies have no bearing on resistance management for tobacco budworm (TBW), *Heliothis virescens*, and pink bollworm (PBW), *Gossypiella pectinophora*, to Bollgard or Bollgard II cotton.

This study is "supplemental" (partially acceptable) and may be upgraded to "acceptable" if the following recommendations described below are addressed.

RECOMMENDATIONS

1. Marcus et al. (2004) found that CBW larvae (late instar larve (L4-L5)) in North Carolina Bollgard and non-Bollgard plots in North Carolina were half as susceptible to Cry1Ac than were populations generated from non-Bollgard cotton survivors in the F1 generation. Additional work beyond that of Marcus et al. (2004) examining the genetics of Cry1Ac tolerance in the F1 generation (whatever its

source) is recommended.

2. The approved protocols (March 14, 2002) for pyrethroid overspray studies included plans to generate a Cry1Ac-resistant CBW colony and use it to investigate the genetic basis for cotton bollworm survival on Bollgard cotton. However, this work could not be completed for inclusion in Monsanto's submission (Greenplate, 2004). It is recommended that the Cry1Ac-resistant CBW colony work be completed.

4. It is recommended that Gustafson et al. (2004) model be refined (or another appropriate resistance management model) using the average pyrethroid efficacy value against CBW calculated based on all the field studies conducted in all four states (North Carolina, Louisiana, Mississippi, and South Carolina) as the new parameter value rather than values strictly from Brickle et al. (1999). If there is no statistical difference in the irrigated and non-irrigated plots then these results may be combined.

BACKGROUND

On September 29, 2001, EPA approved an amendment to Bollgard® cotton registration (EPA Reg. No. 524-478) extending the registration until September 30, 2006, except for the 5% external, unsprayed refuge option which expires on September 30, 2004. As a condition of this registration, EPA required that Monsanto conduct studies to determine the insect resistance management (IRM) value of pyrethroid oversprays (or other insecticide chemistries) used to control cotton bollworm in conventional and Bollgard cotton. EPA registered Bollgard II® cotton on December 23, 2002 (EPA Reg. No. 524-522) with the same pyrethroid overspray data requirements as Bollgard cotton. Typically, Bollgard cotton is sprayed with a pyrethroid insecticide for CBW control late in the season, although Bollgard cotton is sprayed much less than non-Bollgard cotton. The survival of CBW in Bollgard cotton has been well-established (Mahaffey et al., 1995; Lambert et al., 1997). Bollgard II cotton will not typically be sprayed for lepidopteran control because of its greater efficacy (than Bollgard) against CBW (Jackson et al. 2003a). The results from the field research can be used to provide parameters for a resistance prediction model (e.g., Caprio, 1998a and b; Storer, 2003; Gustafson et al., 2004)

Monsanto submitted a proposed protocol on December 1, 2001 (EPA Review, Matten, 2002) and a final protocol on March 14, 2002. EPA reviewed these protocols and found the final protocol to be acceptable. EPA required that Monsanto submit an interim progress report on the studies described in the protocols by March 15, 2003 and a final report by March 15, 2004. The report is reviewed here.

Two approaches were taken in 2002 to examine the efficacy of pyrethroid oversprays on CBW populations in Bollgard cotton.

1. Field studies. Field studies were conducted in North Carolina, South Carolina, Mississippi, and Louisiana to assess the efficacy of pyrethroid insecticides on CBW feeding on Bollgard cotton. Large larvae (L4-L5) surviving in Bollgard cotton plots were collected at some locations and allowed to

pupate to obtain an estimate of the reduction in adult emergence from a pyrethroid insecticide overspray in Bollgard cotton. These four states represent a range of cotton production conditions across the Cotton Belt. Results can be used to provide parameter values for mathematical prediction models for resistance evolution.

2. Laboratory and greenhouse studies. The approved research protocol stated that laboratory and greenhouse studies will be conducted to determine if Cry1Ac-resistant CBW (laboratory colonies) are more, less or equally susceptible to pyrethroid insecticides, and to determine if there is a genetic basis for CBW survival in Bollgard cotton.

3. Interim results. Monsanto provided EPA with the interim progress report on March 13, 2003 containing results of field studies conducted in 2002 and the status of the laboratory and greenhouse studies. No results from the laboratory and greenhouse studies were available for inclusion in the interim report. EPA reviewed the interim progress report and determined it was "acceptable" (Matten, 2003).

Preliminary results from the North Carolina and Louisiana field studies that pyrethroid oversprays are more effective at controlling CBW in Bollgard cotton fields than in non-Bollgard cotton fields. Studies in Mississippi and South Carolina did not provide meaningful results due to low pest pressure and were discontinued in 2003. Threshold levels of CBW populations were never reached in any of the plots to warrant treatment with pyrethroids.

In the final report, Monsanto included results of the field studies conducted in North Carolina and Louisiana. In addition, field studies on pyrethroid efficacy in Bollgard cotton in years prior to 2002 in South Carolina and Mississippi available in the literature are summarized by Monsanto. Efficacy of Cry1Ac and pyrethroid chemistry against progeny of CBW survivors of Bollgard cotton and non-Bollgard cotton were tested in F1 and F2 generations in the lab of Dr. J.R. Bradley at North Carolina State University (NCSU) and results are discussed in the report, as well as data collected in 2002 in South Carolina on the efficacy of pyrethroids against Bollgard cotton survivors. The status of work at Auburn University to generate Cry1Ac-resistant CBW to test their responses to pyrethroids is provided.

REVIEW

1. Field studies.

Field studies were conducted in North Carolina, South Carolina, Mississippi, and Louisiana to assess the efficacy of pyrethroid insecticides on CBW feeding on Bollgard cotton. These locations represent a range of insect pressure, agronomic and environmental conditions across the cotton belt. At each location, the treatments were replicated (where possible) under irrigated and non-irrigated conditions (separate fields). These field trials depended on naturalCBW infestation; thus, results varied across locations. The results of these field research studies have been published in the literature or have been submitted as reports to Monsanto (see Appendices 1-7, Greenplate, 2004). The percent insecticide control values and total number of adults (insecticide-treated versus non-treated) can then be used to develop reliable parameters for the mathematical models (e.g., Gustafson et al., 2004).

North Carolina

In 2001-2002, North Carolina field trials were conducted on both irrigated and non-irrigated cotton (conventional, Bollgard, and Bollgard II) plots (Jackson et al., 2003a, Appendix 1 in Greenplate, 2004; Jackson et al., 2002, Appendix 2 in Greenplate, 2004; Jackson et al., 2003b, Appendix 3 in Greenplate, 2004). Results are discussed below.

The efficacy of pyrethroid oversprays was evaluated by examining larval infestation and damage levels in squares/bolls measured across five test sites **(Table 1)**. Pyrethroid-treated Bollgard and Bollgard II and untreated Bollgard II plots all showed statistically equivalent reductions in percent infested squares and bolls and percent damaged squares and bolls. These three treatments showed statistically significant reductions in percentages of square and boll infestation and percentages of square and boll damage when compared to pyrethroid-treated and untreated conventional plots and untreated Bollgard plots. The addition of pyrethroid oversprays effectively reduced the percentages of infestation in bolls and squares and percentages of square and boll damage for conventional cotton and Bollgard cotton by approximately 70 to 90%, but not for Bollgard II cotton. There was always a greater reduction in percentages of infested squares and bolls and percentages of damaged squares, 78 to 92%, due to pyrethroid oversprays in Bollgard, versus 72 to 80%, due to pyrethroid oversprays in conventional cotton (see Table 1 in Greenplate, 2004).

Table 1. Mean (SE) percentage of squares infested by bollworm larvae and damaged squares; and percentage of bolls containing live (L4 and L5) bollworm larvae and damaged bolls for pyrethroid-treated and untreated subplots of Bollgard, Bollgard II, and non-Bollgard (Conventional) cotton averaged across five test sites (2001 and 2002) in North Carolina (Jackson et al., 2003a).

Cotton Type	Insecticide regime	Percentage squares infested w/larvae[1]	Percentage damaged squares[1]	Percentage bolls infested w/ larvae[1]	Percentage damaged bolls[1]
Conventional (DP50)	Untreated	12.8 (1.403) a	44.3 (3.042) a	14.2 (1.865) a	63.0 (3.601) a
Conventional (DP50)	Pyrethroid-treated	2.5 (0.542) b	13.1 (1.103) b	4.3 (0.520) b	17.6 (1.343) b
Bollgard (DP50B)	Untreated	1.3 (0.287) b	6.6 (1.207) c	4.5 (0.687) b	12.9 (1.437) b
Bollgard (DP50B)	Pyrethroid-treated	0.1 (0.083) c	0.8 (0.264) d	0.6 (0.130) c	2.9 (0.465) c

Cotton Type	Insecticide regime	Percentage squares infested w/larvae[1]	Percentage damaged squares[1]	Percentage bolls infested w/ larvae[1]	Percentage damaged bolls[1]
Bollgard II (DP50BX)	Untreated	0.1 (0.083) c	0.1 (0.083) d	0.4 (0.120) c	1.5 (0.305) c
Bollgard II (DP50BX)	Pyrethroid-treated	0.0 (0.000) c	0.3 (0.155) d	0.0 (0.000) c	0.2 (0.071) c

[1]Means within a column followed by the same letter are not significantly different according to Fisher's Protected LSD (*P* # 0.05).

Jackson et al. (2002) (Appendix 2 in Greenplate, 2004) report that pyrethroid oversprays reduced bollworm production at every life stage measured (4th-5th instar larvae, pupae, and adults) in one non-irrigated site in 2001**(Table 2)**. Similarly, in 2002 **(Table 3)**, combined results from four sites: two irrigated and two non-irrigated, showed significant reduction in bollworm production at every life stage measured, with significantly greater reductions in Bollgard and Bollgard II cotton plots than in non-Bollgard cotton plots (Jackson et al., 2003b) (Appendix 3 in Greenplate, 2004).

Table 2. Estimated mean (SE) effects of pyrethroid oversprays on bollworm production in Bollgard, Bollgard II, and non-Bollgard (Conventional) cotton plots at one non-irrigated site in North Carolina in 2001 across 3 sample dates. (Jackson et al., 2002).

Cotton Type	Life Stage	Untreated[1]	Pyrethroid-Treated[1]	Percentage Reduction Due to Pyrethroid Sprays
Conventional (DP50)	Large Larvae	9,972 (2,367)	4587 (1,048)	54.0
Bollgard (DP50B)	Large Larvae	2,080 (495)	306 (170)	85.3
Bollgard II (DP50BX)	Large Larvae	156 (105)	77 (77)	50.6
Conventional (DP50)	Pupae	9,972 (2,367) a	4,451 (1,048) a	55.4
Bollgard (DP50B)	Pupae	518 (167) b	160 (108) b	69.1
Bollgard II (DP50BX)	Pupae	156 (105) c	0 (0) c	100.0
Conventional (DP50)	Adults	9,972 (2,367) c	3,482 (856) c	65.1

Cotton Type	Life Stage	Untreated[1]	Pyrethroid-Treated[1]	Percentage Reduction Due to Pyrethroid Sprays
Bollgard (DP50B)	Adults	298 (128) c	87 (87) c	70.8
Bollgard II (DP50BX)	Adults	156 (105) c	0 (0) c	100.0

[1] Means within the same column followed by the same letter are not significantly different, LS Means (*P* # 0.05).

Table 2. Estimated mean (SE) effects of pyrethroid oversprays on bollworm production under high bollworm pressure in Bollgard, Bollgard II, and non-Bollgard (Conventional) cotton plots at one non-irrigated site in North Carolina in 2002 across 3 sample dates. (Jackson et al., 2003b).

Cotton Type	Life Stage	Untreated[1]	Pyrethroid-Treated[1]	Percentage Reduction Due to Pyrethroid Sprays
Conventional (DP50)	Large Larvae	34,833 (5,408)	13,208 (1,980)	62.1
Bollgard (DP50B)	Large Larvae	26,775 (3,925)	2,856 (746)	89.3
Bollgard II (DP50BX)	Large Larvae	2,471 (760)	0 (0)	100.0
Conventional (DP50)	Pupae	29,796 (4,671)	7,814 (1,302)	73.8
Bollgard (DP50B)	Pupae	23,264 (3,396)	1,827 (478)	92.1
Bollgard II (DP50BX)	Pupae	2,167 (639)	0 (0)	100.0
Conventional (DP50)	Adults	26,172 (4,245) a	5,714 (1,073) b	78.2
Bollgard (DP50B)	Adults	15,777 (2,504) ab	999 (359) c	93.7
Bollgard II (DP50BX)	Adults	1,067 (435) c	0 (0) c	100.0

[1] Means followed by the same letter are not significantly different, Fisher's Protected LSD (*P* # 0.05).

Louisiana

In Louisiana, efficacy of the pyrethroid insecticide (Karate Z used at a rate of 0.033 lb a.i./acre per application) against CBW was determined on non-Bollgard and Bollgard cotton under non-irrigated and irrigated conditions (Leonard, 2003 (Appendix 5 in Greenplate, 2004). Results of the studies were reported as averages across locations and irrigated and non-irrigated treatments. Efficacy against natural CBW infestations was measured by counting percent damaged bolls. Results from the 2002 studies indicate that there were no differences between irrigated and non-irrigated treatments so results were combined. Data were analyzed with ANOVA, and means were separated according to DMRT. Specific insect species determinations showed that approximately two-thirds of the heliothine insect infestations (range was 45% to 85% across all five sampling dates) of cotton were due to CBW (the other one-third included TBW). Results from 2002 indicated pyrethroid oversprays reduced both the percentages of heliothine damaged bolls and boll infestations and reductions were greater in Bollgard cotton than in non-Bollgard cotton (**Table 4).** Results show that there was a seasonal average of 25.2% of the unsprayed non-Bollgard cotton bolls damaged by heliothine infestation. Following pyrethroid oversprays, heliothine damage was reduced to an average of 11.25% damaged bolls in non-Bollgard cotton, a 55.4% reduction. In contrast, unsprayed Bollgard fields had a 3.91% seasonal average of heliothine damage that was reduced to 1.63% after treatment with pyrethroid oversprays, a 58.3% reduction. Unsprayed non-Bollgard cotton suffered a seasonal average of 6.75% bolls infested with larvae. This infestation level was reduced 4.02% after pyrethroid oversprays, a 40.4% reduction. In contrast, unsprayed Bollgard fields had a 1.1% infestation level. This damage was reduced to 0.4% following pyrethroid oversprays, a 63.6% reduction. There was a statistically significant difference in the percent larvae recovered in bolls (far fewer) in Bollgard cotton sprayed with pyrethroid than in either unsprayed Bollgard cotton or non-Bollgard sprayed or unsprayed cotton. Sprayed and unsprayed Bollgard and non-Bollgard cotton yields with and without pyrethroid overspray treatment were compared (see Table 1 in Leonard, 2003). Sprayed non-Bollgard cotton yielded 639 lb per acre versus 181 lb per acre for unsprayed non-Bollgard cotton. The sprayed Bollgard cotton treatment yielded 1448 lb per acre versus 855 lb per acre for the unsprayed Bollgard cotton. As seen in the North Carolina studies, reductions by pyrethroid oversprays in Bollgard cotton plots were greater than reduction by pyrethroid oversprays in non-Bollgard cotton plots.

Table 4. Mean percentages of larvae-infested bolls and damaged bolls in a Louisiana field study in 2002 (Leonard, 2003). Because there were no significant differences in irrigation and non-irrigated plots, results were combined. (Table reprinted from p. 14, Greenplate, 2004)

% Bolls Infested with Larvae			
	Sprayed	**Unsprayed**	**% Reduction due to spray**
Bollgard	0.4	1.1	63.6
non-Bollgard	4.02	6.75	40.4
% Heliothine Damaged Bolls			
	Sprayed	**Unsprayed**	**% Reduction due to spray**

Bollgard	1.63	3.91	58.3
non-Bollgard	11.25	25.2	55.4

Mississippi

A study was initiated at the Delta Research and Extension Center, Mississippi State University in 1999 and repeated in 2000 and 2001 to evaluate Bollgard II cotton for lepidopteran control in comparison with two Bollgard cottons and a conventional non-transgenic Bt *(Bacillus thuringiensis*) cotton. Results for the 2001 growing season are published in Harris et al. (2002) (Appendix 6 in Greenplate, 2004). Cotton varieties were tested under lepidopteran insecticide regimens that were sprayed (cyhalothrin, a synthetic pyrethroid) or unsprayed. Tarnished plant bugs were sprayed with imidacloprid and oxamyl. Observations in each plot included whole plant samples (25), sweep-net (15 in) samples (3), drop-cloth samples (3 samples = 18 row ft.), visual observations of foliage on 30 row ft., visual ratings of natural enemies, visual rating of cotton aphid and whitefly infestations, terminals (25), squares (50), blooms (25), young bolls less than 2 cm dia (50), old bolls greater than 2 cm dia (50), and bolls with bloom tags, i.e., stuck petals (50). Data were recorded for the following insect species: cotton bollworm, *Helicoverpa zea* (Boddie), tobacco budworm, *Heliothis virescens* (F.); beet armyworm, *Spodoptera exigua* (Hübner); fall armyworm, *Spodoptera frugiperda* (J.E. Smith); saltmarsh caterpillar, *Estigmene acreae* (Drury); cabbage looper, *Trichoplusia ni* (Hübner); tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois); green stink bug, *Acrosternum hilare* (Say); brown stink bug, *Euschistus servus* (Say); cotton aphid, *Aphis gossypii* (Glover); and whitefly (predominantly banded winged whitefly), *Trialeurodes abutilonea* (Haldeman). This review focuses on those results pertaining to CBW and TBW larval damage and infestation.

Larval counts were made four or five times in pyrethroid-sprayed (five sprays) and unsprayed Bollgard II, Bollgard, and non-Bt conventional cotton plots. During the seven-week larval sampling period, adult trapping data indicated the presence of approximately 59% CBW and 41% TBW across this period (see Table 1 in Harris et al., 2002). Means for larval counts in sprayed and unsprayed Bollgard II, Bollgard, and non-Bt conventional cotton plots appear in **Table 5.** As seen in the other field studies for North Carolina and Louisiana, there were greater larval reductions caused by pyrethroid oversprays in Bollgard and Bollgard II cotton plots than in non-Bt cotton plots. Unsprayed Bollgard II had virtually no CBW/TBW damage.

Table 5. Effects of pyrethroid sprays on cotton bollworm/tobacco budworm larval numbers in whole plants and selected plant tissues in Mississippi in 2001 (Harris et al., 2002).

Mean # Larvae per 25 Whole Plants			
	Sprayed	**Unsprayed**	**% Reduction due to spray**

non-Bollgard (DP 5415)	3.5	3.2	-9.4
Bollgard (NuCotn 33B)	0.58	0.52	-11.5
Bollgard (PM 1218BR)	0.28	1.02	72.6
Bollgard II (DPLX01T21 BGII)	0.0	0.15	100.0
Mean # Larvae per Sample*			
	Sprayed	**Unsprayed**	**% Reduction due to spray**
non-Bollgard (DP 5415)	6.0	11.5	47.8
Bollgard (NuCotn 33B)	0.28	1.02	72.6
Bollgard (PM 1218BR)	1.1	3.8	71.1
Bollgard II (DPLX01T21 BGII)	0.0	0.06	100.0

*25 terminals + 50 squares + 25 blooms + 50 young bolls + 50 old bolls + 50 bloom tags

South Carolina

In 1998 and 1999 similar studies to those conducted in North Carolina, Louisiana, and Mississippi were conducted in South Carolina (Brickle et al., 2001) (Appendix 7 in Greenplate, 2004). These studies were unique in that pyrethroid (lambda-cyhalothrin) applications and five other insecticides, spinosad, thiodicarb, indoxacarb, chlorfenapyr, and enamectin benzoate, were evaluated against the CBW at three different application rates under non-irrigated and irrigated conditions. Results for non-irrigated Bollgard and non-Bollgard cotton in 1998 and 1999 are in **Table 6.** For both years in non-irrigated cotton, pyrethroid oversprays, regardless of the application rate, significantly reduced infestation levels in both non-Bollgard and Bollgard cotton plots with relative greater efficacy in Bollgard cotton as was observed in field studies in the North Carolina, Louisiana, and Mississippi. Results for irrigated Bollgard and non-Bollgard cotton in 1999 are in **Table 7**. Pyrethroid-sprayed non-Bollgard cotton exhibited slightly better proportional reduction of bollworms at the two highest pyrethroid rates than Bollgard cotton. Brickle et al. (2001) speculate that this might be due to excessive soil moisture and vegetative growth or intrinsic differences in Bt expression. These authors do not recommend reduced rates of lambda-cyhalothrin, spinosad, or thiodicarb in irrigated Bollgard cotton systems, but these low rates could be used for non-irrigated Bollgard cotton systems.

Table 6. Effects of pyrethroid oversprays on non-irrigated Bollgard and non-Bollgard cotton in South Carolina in 1998 and 1999 (Brickle et al., 2001).

1998 Non-Irrigated Trial		**Mean # Larvae per 15m Row**		
	Rate (kg lambda-cyhalothrin/ha)	**Sprayed**	**Unsprayed**	**% Reduction due to spray**
non-Bollgard (DP 5415)	0.028	16.0	50.7	68.5
non-Bollgard (DP 5415)	0.015	17.6	50.7	65.3
non-Bollgard (DP 5415)	0.007	28.6	50.7	43.7
Bollgard (NuCotn 33B)	0.028	0.0	15.0	100.0
Bollgard (NuCotn 33B)	0.015	3.1	15.0	79.4
Bollgard (NuCotn 33B)	0.007	3.3	15.0	77.8
1999 Non-Irrigated Trial		**Mean # Larvae per 15m Row**		
	Rate (kg lambda-cyhalothrin/ha)	**Sprayed**	**Unsprayed**	**% Reduction due to spray**
non-Bollgard (DP 5415)	0.028	19.0	140.1	86.4
non-Bollgard (DP 5415)	0.015	39.4	140.1	71.9
non-Bollgard (DP 5415)	0.007	68.4	140.1	51.2
Bollgard (NuCotn 33B)	0.028	1.4	26.6	94.6
Bollgard (NuCotn 33B)	0.015	3.8	26.6	85.7
Bollgard (NuCotn 33B)	0.007	6.7	26.6	75.0

Table 7. Effects of pyrethroid oversprays on irrigated Bollgard and non-Bollgard cotton in South Carolina in 1999 (Brickle et al., 2001).

1999 Irrigated Trial		Mean # Larvae per 15m Row		
	Rate (kg lambda-cyhalothrin/ha)	**Sprayed**	**Unsprayed**	**% Reduction due to spray**
non-Bollgard (DP 5415)	0.028	8.9	129.8	93.1
non-Bollgard (DP 5415)	0.015	22.0	129.8	83.0
non-Bollgard (DP 5415)	0.007	74.4	129.8	42.7
Bollgard (NuCotn 33B)	0.028	5.4	41.7	87.1
Bollgard (NuCotn 33B)	0.015	17.3	41.7	58.6
Bollgard (NuCotn 33B)	0.007	18.4	41.7	55.7

2. Laboratory and greenhouse studies.

The approved protocols (March 14, 2002) for pyrethroid overspray studies included plans to generate a Cry1Ac-resistant CBW colony and to use it to investigate the genetic basis for CBW survival on Bollgard cotton. However, this work could not be completed for this report. It is recommended that the Cry1Ac-resistant CBW colony work be completed.

North Carolina.

Surviving heliothine larvae (late instar larve (L4-L5)) in plots in North Carolina were collected from Bollgard cotton and non-Bollgard cotton plots and their offspring were tested in the laboratory for susceptibility to the Cry1Ac protein and to a pyrethroid (cypermethrin) (Marcus et al., 2004, Appendix 4 in Greenplate, 2004). Results showed that CBW populations generated from Bollgard cotton survivors were half as susceptible to Cry1Ac than were populations generated from non-Bollgard cotton survivors in the F1 generation, but this difference disappeared in the F2 generation **(Table 8)**. There was no statistical difference between these two groups in terms of their susceptibility to cypermethrin at either the F1 or F2 generations **(Table 9)**.

Table 8. LC_{50} values for Cry1Ac against progeny of bollworm survivors from Bollgard and non-Bollgard cotton (Marcus et al., 2004) (Reprinted from p. 17, Greenplate 2004)

Strain	Generation	LC_{50} (mg/mL)	95% confidence limits	
			Upper	Lower
Non-Bt	F1	16.3	21.8	12.3
Bollgard	F1	30.6	37.9	25.5
Non-Bt	F2	13.2	27.9	7.41
Bollgard	F2	15.6	19.6	12.8

Table 9. LC_{50} values for technical grade cypermethrin against progeny of bollworm survivors from Bollgard and non-Bollgard cotton (Marcus et al., 2004) (Reprinted from p. 17, Greenplate, 2004)

Strain	Generation	LC_{50} (mg/mL)	95% confidence limits	
			Upper	Lower
Non-Bt	F1	0.623	0.819	0.504
Bollgard	F1	0.806	2.82	0.528
Non-Bt	F2	0.179	0.217	0.137
Bollgard	F2	0.150	0.191	0.069

South Carolina

Brickle et al. (2001) (Appendix 7 in Greenplate, 2004), as part of their 1998 and 1999 South Carolina study (discussed above), evaluated several insecticides for efficacy against surviving CBW larvae. Only third instar larvae from Bollgard cotton had a LC_{50} that was half that of non-Bollgard survivors, but this difference was not statistically significant. Pyrethroid (lamda-cyhalothrin) was the only insecticide with a lower LC_{50} value for larvae collected from Bollgard cotton. Spinosad, thiocarb, and indoxacarb insecticides all had larger LC_{50} values for Bollgard cotton survivors when compared to non-Bollgard survivors.

Discussion and Conclusions

1. Field Studies

Results from field studies conducted in 1998-2002 in North Carolina, Louisiana, Mississippi, and South

Carolina show that CBW larvae surviving in Bollgard and Bollgard II (the few that are there) are effectively controlled with supplemental pyrethroid spray treatments. In almost all cases shown, regardless of the evaluation criteria (i.e., larval counts, plant damage assessments (squares and bolls), larval/pupal/adult production per acre)) or irrigation regime, pyrethroid oversprays significantly reduced larval damage, infestation levels, and gross insect production in non-Bollgard, Bollgard, and Bollgard II cotton plots with relative greater efficacy in Bollgard and Bollgard II cotton (Tables 1-6). The only exception to this involved larval counts recorded in one irrigated field study in South Carolina in 1999 (Table 7) in which pyrethroid sprays reduced larval infestation levels to a greater extent in non-Bollgard cotton than Bollgard cotton. Brickle et al. (2001) suggest that this may be due to excessive soil moisture or vegetative growth or intrinsic differences in *Bt* (*Bacillus thuringiensis*) expression. Additional field studies examining the pyrethroid efficacy in irrigated and non-irrigated Bollgard and non-Bollgard cotton plots are recommended in South Carolina to determine whether pyrethroid efficacy is truly greater in irrigated non-Bollgard cotton than in irrigated Bollgard cotton.

Greenplate (2004) offered several possible explanations based on the literature as to why pyrethroid efficacy is greater in Bollgard cotton than in non-Bollgard cotton. Brickle et al. (2001) suggest that pyrethroid sprays may be more effective when directed against Bollgard cotton survivors than non-Bollgard cotton survivors because the larvae are already stressed by partial Cry1Ac intoxication and thus are more vulnerable to pyrethroids. Gore et al. (2002) report that bollworm larvae move much farther vertically on a Bollgard plant than on a non-Bollgard plant. These authors and Greenplate (1998) speculate that CBW larvae may be able to detect the Cry1Ac toxin and avoid it by moving vertically on the Bollgard plant. Greenplate (2004) concludes that "if a pyrethroid has greater effectiveness in Bollgard cotton because it encounters a Cry1Ac-compromised larva and/or if it has a greater chance of contacting a more mobile larva, the result will be a relative greater effectiveness in Bollgard cotton over conventional cotton." This result is consistently confirmed by all of the field studies in North Carolina, Louisiana, Mississippi and non-irrigated cotton in South Carolina. EPA agrees with the Greenplate (2004) analysis.

Greenplate (2004) does not discuss the pyrethroid efficacy on Bollgard II cotton although it has included the studies in the Appendices. EPA mandated the same pyrethroid overspray data requirements for both the Bollgard registration (EPA Reg. No. 524-478) and the Bollgard II registration (EPA Reg. No. 524-522). The requirement states that "research studies to determine the IRM value of different insecticide chemistries likely to be used against the cotton bollworm in conventional and transgenic Cry1Ac *Bt* cotton (irrigated and non-irrigated, side by side field trials)" be conducted. "Any potential effects must be related to survival of putative Cry1Ac *Bt*-resistant cotton bollworm and effective refuge size." Cry1Ac is a protein expressed in both Bollgard and Bollgard II cotton. Bollgard II cotton also expresses the Cry2Ab2 protein. EPA has included in this review the Bollgard II data from field studies conducted in North Carolina by Jackson et al. (2002, 2003a and b, Appendices 1-3 in Greenplate, 2004) and in Mississippi by Harris et al. (2002) (Appendix 6 in Greenplate, 2004). Percentage squares and bolls infested and percentage damaged squares and bolls were not statistically different in Bollgard treated and Bollgard II untreated and treated plots. Bollgard II expresses a high level of both Cry1Ac and Cry2Ab2 season-long to protect fruit from CBW feeding;

while, Bollgard expresses only a moderate level of Cry1Ac and does not protect fruit from CBW feeding season-long (see EPA review of Bollgard II IRM, Matten, 2002). Results from North Carolina and Mississippi field studies suggest that pyrethroid oversprays will likely not be necessary for CBW in Bollgard II cotton fields as they were for Bollgard cotton fields. That is, it is less likely that economic thresholds will be reached in Bollgard II fields for lepidopteran control than in Bollgard and conventional fields.

2. Laboratory and greenhouse studies

North Carolina laboratory studies (Marcus et al., 2004) showed that progeny of Bollgard cotton survivors were half as susceptible to Cry1Ac than were populations generated from non-Bollgard cotton survivors in the F1 generation, but this difference disappeared in the F2 generation **(Table 8)**. Tolerance to Cry1Ac in the F1 generation was not statistically significant and may not truly exist. The authors suggest that there might be a "negative maternal effect" caused by stressed parents feeding on artificial diet to produce more fit offspring. The progeny of Bollgard and non-Bollgard cotton survivors showed no statistical difference in their susceptibility to cypermethrin at either the F1 or F2 generations **(Table 9)**. The Cry1Ac tolerance (whatever its source) in the F1 generation is independent of pyrethroid susceptibility. It can be inferred that pyrethroid efficacy would be unaffected by increased CBW tolerance. Marcus et al. (2004) suggest that lack of any statistically significant difference may be due to the low statistical power of the experiment given there was such a low level of tolerance to the Cry1Ac toxin in the Bollgard strain. Authors indicate that they are working to select for a CBW strain that has substantial Cry1Ac resistance to provide more rigorous results.

The studies at Auburn University to generate a Cry1Ac-resistant (laboratory) CBW colony and test its response to pyrethroid insecticides could not be completed. It is recommended that these studies be continued and these data be used to determine if there is a genetic basis for CBW survival on Bollgard cotton.

3. Modeling

Monsanto developed an insect resistance management model (Gustafson et al., 2004; original report dated September 10, 2001 submitted as a public comment to EPA Docket OPP-00678B). This model was developed based on Caprio (1998) to demonstrate the sensitivity of the model output (i.e., years to resistance) to insecticidal oversprays of Bollgard cotton fields and the utilization of alternate hosts as natural refugia. The Gustafson et al. (2004) model is reviewed separately.

Currently the Gustafson et al. (2004) model is parameterized with pyrethroid efficacy values from Brickle et al. (2001) because there was only limited data available. Using data from Brickle et al. (2001), the Gustafson et al. (2004) model predicted that a greater than ten-fold increase in the time to resistance (resistance allele frequency is greater than 0.5) for CBW when Bollgard cotton was sprayed with pyrethroids. Research summarized in Greenplate et al. (2004) provides additional data to incorporate into population genetics models, e.g., Gustafson et al. (2004). They conclude that "the overwhelming agreement of the data on pyrethroid efficacy in Bollgard cotton, collected from various locations in the U.S. Cotton Belt, will likely provide modeling results similar to those generated by Gustafson et al. (2004) and strongly support the utility of pyrethroid oversprays in Bollgard cotton to significantly delay resistance development in CBW populations." The purpose of EPA requesting that empirical data be collected regarding pyrethroid overspray efficacy across several different cotton production systems was that these data would be used to more precisely parameterize the Gustafson et al. (2004) model or other resistance management models. As stated in EPA's *Bacillus thuringiensis (Bt*) Plant-Incorporated Protectants Biopesticide Registration Action Document (EPA, 2001), "Once this information has been gathered, the registrant must refine or construct new resistance management models for appropriate cotton producing areas in the U.S. (i.e., areas where *Helicoverpa zea* typically exceeds economic thresholds on *Bt* cotton)." Monsanto did not refine the Gustafson et al. (2004) model or any other resistance management model with the field study data that were analyzed and described in Greenplate et al. (2004).

Results from field studies in North Carolina (Jackson et al. (2002, 2003a and b)), Mississippi (Harris et al., 2002), and Louisiana (Leonard, 2003) show a wide range of values for pyrethroid efficacy against CBW on irrigated or non-irrigated Bollgard (55% to 94%) and non-Bollgard cotton (0% to 78%) Brickle et al. (2001) report that in field studies conducted in South Carolina that values for pyrethroid efficacy against CBW ranged from 75% to 100% in dryland Bollgard cotton and 51% to 86% in dryland non-Bollgard cotton with the highest efficacy achieved using the highest pyrethroid application rate (0.028 kg ai/ha lambda-cyhalothrin). However, the irrigated study in Brickle et al. (2001) showed that pyrethroid efficacy was lower in Bollgard cotton (59% and 87% at the two highest application rates, respectively) than in non-Bollgard cotton (83% and 93% at the two higheset application rates, respectively). There are questions regarding the irrigated study of pyrethroid efficacy against CBW. This was the only field study that showed that pyrethroid efficacy against CBW was greater in irrigated non-Bollgard cotton than in irrigated Bollgard cotton. Currently the Gustafson et al. (2004) resistance management model for Bollgard cotton is parameterized with pyrethroid efficacy values from Brickle et al. (2001). It is recommended that Gustafson et al. (2004) model be refined (or another appropriate resistance management model, e.g., Storer, 2003; Caprio, 1998b) using the average pyrethroid efficacy value against CBW based on all the field studies conducted in all four states (North Carolina, Louisiana, Mississippi, and South Carolina) as the new parameter value rather than values strictly from Brickle et al. (1999). If there is no statistical difference in the irrigated and non-irrigated plots then these results may be combined.

Based on the North Carolina (Jackson et al. 2002, 2003 a and b) and Mississippi (Harris et al. 2002) field studies discussed above, Bollgard II cotton fields are unlikely to be sprayed with pyrethroids for CBW control as are Bollgard and conventional cotton. Pyrethroid sprays on Bollgard II plots do not provide a statistically significant difference in reduction of CBW infestation or damage from untreated Bollgard II cotton fields or from treated Bollgard cotton fields. Thus, pyrethroid oversprays, as a parameter in the Gustafson et al. (2004) resistance model should not significantly impact the model output for CBW on Bollgard II cotton. Pyrethroid oversprays are not an important parameter in the Gustafson et al. (2004) model for CBW resistance management to Bollgard II cotton.

Summary

Pyrethroid oversprays in Bollgard cotton fields will increase the level of control of CBW, delay the evolution of resistance (see Gustafson et al.'s (2004) model predictions), and support the continuation of the 5% external, unsprayed, structured refuge requirement. The vast majority of field studies conducted in North Carolina, Louisiana, Mississippi, and South Carolina indicate that pyrethroid oversprays caused a greater percent reduction in cotton bollworm (CBW) infestation levels or boll/square/plant damage in Bollgard cotton fields than in non-Bollgard cotton fields whether irrigated or non-irrigated. These four states represent a range of cotton production conditions across the Cotton Belt. Laboratory and greenhouse studies have not uncovered a reason for this fitness difference. Results of the field studies should be used to revised the parameter estimations in Gustafson et al.'s (2004) model for mathematical prediction of CBW resistance evolution.

Based on field studies conducted in Mississippi and Louisiana, Bollgard II will not likely be treated with pyrethroid oversprays for CBW control. Thus, pyrethroid oversprays will not be an important parameter in the Gustafson et al. (2004) model for CBW resistance management to Bollgard II cotton.

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