EFFICACY OF DIMILIN® (DIFLUBENZURON) AND TRANSGENICBt COTTON ON SEVERAL LEPIDOPTERAN SPECIES

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Abstract

A laboratory bioassay was used to evaluate the effect of Dimilin in combination with transgenic cotton (Bollgard™), which incorporates the genes to manufacture the Bacillus thuringiensis (Bt) endotoxin, on several lepidopteran species. Bollgard was highly effective in minimizing leaf feeding and causing mortality of third instar larvae of tobacco budworm (Heliothis virescens), cotton bollworm (Heliocoverpa zea) and soybean looper (Pseudoplusia includens), but was less efficacious for beet armyworm (Spodoptera exigua) and fall armyworm (Spodoptera frugiperda). For beet armyworm Dimilin (diflubenzuron) in combination with Bt cotton was more effective in reducing larval leaf feeding and increasing larval mortality than either Dimilin or Bt cotton alone. Instances of synergistic responses to Dimilin and Bt cotton for both larval feeding and mortality were found for this species. Although Dimilin alone was highly effective on fall armyworm, the advantage of Dimilin on Bt cotton over Dimilin on non-Bt cotton was not evident for this species.

Introduction

Purified endotoxin proteins of Bacillus thuringiensis (Bt) have been shown to be active in controlling several species of Lepidoptera (Hofte and Whiteley, 1989), and foliar applications of Bt have demonstrated limited control of boll feeding insects on cotton (e.g. Shafique and Luttrell, 1992). Transgenic cotton which synthesizes the endotoxin proteins of Bt has recently became commercially available for the first time in 1996. The Bt genes in transgenic cotton are expressed in leaves, squares and bolls (Perlak et al., 1990). Whether evaluated in a bioassay, in a greenhouse, or small and large plot field trials, several Bt cotton lines have been shown to be efficacious against tobacco budworm (Heliothis virescens), cotton bollworm (Heliocoverpa zea), and pink bollworm (Pectinophora gossypiella) (Benedict et al., 1992; Jenkins et al., 1993; Mahaffey et al., 1994; Mascarenhas et al., 1994; Watson, 1995; and others).

Although tobacco budworm is highly sensitive to the endotoxin protein, other lepidopteran species have shown variable susceptibility to it under controlled conditions (Hofte and Whiteley, 1989; MacIntosh et al., 1990; Perlak et al., 1990). In laboratory studies, Stone and Sims (1993) found cotton bollworm to be less susceptible to Bt endotoxin than tobacco budworm. Concomitantly, significant boll damage by cotton bollworm has been found in Bt cotton plots (Mahaffey et al., 1994; Mahaffey et al., 1995). Excellent control of cabbage looper (Trichoplusia ni) has been found with Bt cotton in small field plots (Harris et al., 1996) and Luttrell et al. (1995) found lower looper populations in Bt cotton versus non-Bt cotton. Suppression of beet armyworm (Spodoptera exigua) has been noted (Davis et al., 1995; Harris et al., 1996). Yet Burris et al. (1994) found no differences in leaf area consumed, mortality or pupal weights of beet armyworms when larvae were bioassayed on Bt cotton leaves from the field versus non-Bt leaves. Under low fall armyworm (Spodoptera frugiperda) pressure, Bacheler and Mott (1996) suggest feeding was reduced in Bt cotton. Watson (1995) found Bt cotton used in a bioassay gave activity against third instar saltmarsh caterpillar (Estigmene acrea) larvae.

The insect growth regulator, diflubenzuron (Dimilin), interferes with chitin formation/deposition in susceptible larvae when ingested (Mulder and Gijswijt, 1973). Ingestion of sublethal amounts by larvae can result in weakened pupae and malformed adults (Van Laecke et al., 1989). Chauthani (1978) found that a mixture of diflubenzuron and Bt gave improved mortality of both cabbage looper and tobacco budworm. Conversely, Mohamed et al. (1983) found that a combination of Bt (var. kurstaki) and Dimilin to cotton foliage was antagonistic to controlling first and third instar tobacco budworm. Dimilin appears under some instances to predispose insects to be more susceptible to other insecticides when tank-mixed; Dimilin is also known to be synergistic with several cotton insecticides (Van Laecke and Degheele, 1991; Haynes, 1995).

This study evaluated leaf feeding and larval survival of tobacco budworm, cotton bollworm, beet armyworm, fall armyworm and soybean looper (Pseudoplusia includens) on either non-Bt or Bt transgenic cotton. The potential effects of Dimilin in combination with Bt cotton were also investigated for controlling those species for which Bt cotton alone did not achieve a high level of control.

Materials and Methods

Plants of Stoneville 453 (non Bt) and the Bt (Bollgard™) cotton line, NuCOTN 33B, were individually grown in the greenhouse in 4 inch azalea pots to the 4 true-leaf stage. Prior to spraying with Dimilin 2L, all plants were stripped of foliage except for the 2 most recently expanded leaves. Application method, larval culturing and bioassay description are as reported by McDonald and Weiland (1995). The rates of Dimilin used for a given lepidopteran species were determined in previous experiments with non-Bt cotton (results not shown). These rates resulted in less

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than maximum control of the given insect, thus allowing for determination of any enhanced activity in combination with Bt cotton.

Data were analyzed using Duncan's Multiple Range test (Duncan, 1955) with a significance level of P=0.05.

Results and Discussion

Larvae of three lepidopteran species (tobacco budworm, beet armyworm and fall armyworm) were placed on either Bt transgenic cotton leaves or non-Bt cotton leaves. Feeding activity (Table 1) and survivability (Table 2) were assessed through 5 days of bioassay (DOB). Leaf feeding was minimal after 1 DOB for tobacco budworm on Bt cotton; was not affected by cotton type for beet armyworm; and was reduced approximately 50% on Bt cotton, starting at 2 DOB for fall armyworm (Table 1). Very high mortality had occurred by 2 DOB for tobacco budworm on Bt cotton (Table 2). With beet armyworm, there was no increased mortality on Bt cotton compared with non-Bt cotton. Slight but significantly different mortality occurred for fall armyworm on Bt cotton at both 2 and 3 DOB, but there were no differences thereafter. It was concluded that the Bt cotton possessed high activity on budworm larvae and thus evaluation with Dimilin would not be possible.

The effects of Bt cotton and Dimilin on beet armyworm were explored in two additional evaluations. Three concentrations of Dimilin were investigated. Contrary to results in Table 1, leaf feeding on Bt cotton was reduced for beet armyworm larvae starting at 2 DOB (Table 3); feeding increased through 5 DOB but remained significantly less than on non-Bt cotton. Dimilin at 47 ppm on non-Bt leaves significantly reduced feeding starting at 2 DOB; application of the lower concentrations resulted in little to no reduction in consumption. The combination of Dimilin at either 12 or 3 ppm on Bt cotton gave significantly greater reduction of leaf feeding starting at 3 and 4 DOB, respectively, in comparison to either Dimilin or Bt cotton alone. The response in both instances was synergistic. A similar but additive trend was found for Dimilin at 47 ppm on Bt cotton.

Although leaf feeding by beet armyworm larvae was significantly reduced on Bt cotton starting at 2 DOB (Table 3), mortality was not increased until 5 DOB (Table 4). Dimilin on non-Bt cotton significantly increased mortality starting at 1, 5 and 5 DOB for respective rates of 47, 12 and 3 ppm. The combination of Dimilin at 47 and 12 ppm on Bt cotton gave significantly enhanced mortality starting at 3 and 4 DOB, respectively, versus either Bt cotton or Dimilin alone. The response in both cases was synergistic. A similar but additive trend was found for Dimilin at 3 ppm on Bt cotton.

The bioassay of Dimilin in combination with Bt cotton was repeated (Tables 5 and 6). As noted in the earlier evaluation (Table 3), beet armyworm larvae showed reduced leaf feeding in this second evaluation, starting as early as 1 DOB (Table 5). Again leaf feeding increased through 5 DOB but remained significantly less than on non-Bt-cotton. Dimilin at 47 and 12 ppm on non-Bt cotton significantly reduced feeding starting at 2 and 3 DOB, respectively. The combination of Dimilin at 12 ppm on Bt cotton gave significantly less leaf feeding at 4 DOB, versus either Dimilin or Bt cotton alone. A similar trend was found for Dimilin at 47 and 3 ppm on Bt cotton.

Although leaf feeding was affected by Bt cotton in the first (Table 3) and second evaluation (Table 5), mortality was not altered by type of cotton in the second evaluation (Table 6). The highest rate of Dimilin (47 ppm) on non-Bt cotton did lessen survivability starting a 4 DOB. Although the combination of Dimilin on Bt cotton did not exhibit statistically enhanced mortality over either treatment alone, as found in Table 4, the combination generally gave the greatest mortality when differences in means were found.

Of the three experiments investigating beet armyworm control with Bt cotton versus non-Bt cotton, one showed neither an effect on larval leaf consumption (Table 1) nor on larval mortality (Table 2); one showed effects on both parameters (Tables 3 and 4), and one exhibited effects on leaf consumption (Table 5) but not larval mortality (Table 6). Burris et al. (1994) had determined no effect of Bt cotton on mortality and feeding of beet armyworm. Notwithstanding, suppression of beet armyworm activity by Bt cotton has been reported by Davis et al. (1995) and Harris et al. (1996). Thus there appears to be an undefined influence resulting in inconsistency.

Fall armyworms were also evaluated for control by Bt cotton, Dimilin and the combination of both. As shown in Table 1 and 2, both leaf damage and mortality of larvae were affected by the Bt cotton (Table 7 and 8, respectively). Dimilin on non-Bt cotton reduced leaf feeding starting at 4 DOB for both 47 and 12 ppm (Table 7) and increased mortality starting at 3 DOB and 4 DOB for 47 and 12 ppm, respectively (Table 8). Dimilin on non-Bt cotton at both 47 and 12 ppm gave faster control of fall armyworm than on Bt cotton alone (Table 8). The combination of Bt cotton and Dimilin did not give increased activity over either treatment alone (Tables 7 and 8), contrary to that found with beet armyworm.

The effects of Bt cotton and Dimilin were also assessed on soybean looper larvae (Tables 9 and 10). Although Dimilin at both 748 and 187 ppm affected leaf consumption starting at 3 DOB, Bt cotton affected this parameter at 1 DOB (Table 9). Very little feeding took place after that time, illustrating excellent activity by Bt cotton on soybean looper. Dimilin at 748 and 187 ppm increased larval mortality starting at 2 and 3 DOB, respectively (Table 10). However, Bt cotton gave greater control, either alone or in combination with Dimilin, in contrast to Dimilin alone.

Potential benefits of the combination of Bt cotton and Dimilin were not able to be shown due to the overwhelming activity by Bt in the transgenic cotton.

Results with cotton bollworm (Tables 11 and 12) were similar to those found for soybean looper on Bt cotton. Both prevention of leaf consumption starting at 1 DOB (Table 11) and mortality starting at 2 DOB (Table 12) were determined for Bt cotton on cotton bollworm. Dimilin gave no reduction of leaf consumption throughout the bollworm bioassay (Table 11) with minimal increase in larval mortality at 5 DOB (Table 12). Any benefits of the combination of Bt cotton and Dimilin were again not able to be demonstrated due to the overwhelming Bt activity of the transgenic cotton on the cotton bollworm.

Across bioassays, Dimilin activity on larval mortality was determined from 2 to 5 DOB. This is probably related to the time that larvae were first exposed to the Dimilin and the timing of their molting (Weiland et al., 1996). Mortality occurring closer to 5 DOB was probably related to the low application rates of Dimilin.

Despite some inconsistency of effect of Bt cotton on beet armyworm, in both beet armyworm evaluations there was a significant decrease in leaf feeding for the combination of Bt cotton and several rates of Dimilin, in contrast to either Dimilin alone or Bt cotton alone. Similar treatment responses were found for increasing larval mortality. The combination of Dimilin and Bt cotton resulted in instances of synergistic reduction of leaf feeding and increase of amryworm mortality. This suggests that under field conditons, Dimilin may provide increased control of beet armyworm larvae feeding on Bt cotton than currently provided by Dimilin applied on non-Bt cotton.

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Table 1. Cumulative percent leaf damage by tobacco budworm, beet armyworm and fall armyworm larvae as affected by transgenic Bt cotton (NuCOTN 33B) through 5 days of bioassay.

	Cumulative % leaf damage				
Treatment	1 DOB	2 DOB	3 DOB	4 DOB	5 DOB
Tobacco Budworm					
Stoneville 453	12 a*	13 a	28 a	58 a	84 a
NuCOTN 33B	1 b	2 b	4 b	4 b	8 b
Beet Armyworm					
Stoneville 453	5 a	6 a	8 a	13 a	30 a
NuCOTN 33B	6 a	6 a	6 a	14 a	28 a
Fall Armyworm					
Stoneville 453	16 a	29 a	94 a	151 a	182 a
NuCOTN 33B	12 a	18 b	62 b	110 b	131 b

*Means followed by the same letter within a column for a given species do not significantly differ (P=0.05, Duncan's MRT).

Table 2. Survival of tobacco budworm, beet armyworm and fall armyworm larvae as affected by transgenic Bt cotton (NuCOTN 33B) through 5 days of bioassay (DOB).

	Mean # of Larvae Surviving / 5				
Treatment	1 DOB	2 DOB	3 DOB	4 DOB	5 DOB
Tobacco Budworm					
Stoneville 453	3.4 a*	3.1 a	2.4 a	2.1 a	1.9 a
NuCOTN 33B	3.3 a	0.6 b	0.4 b	0.3 b	0.3 b
Beet Armyworm					
Stoneville 453	4.5 a	4.5 a	4.5 a	4.3 a	3.8 a
NuCOTN 33B	4.9 a	4.9 a	4.5 a	4.1 a	2.6 a
Fall Armyworm					
Stoneville 453	5.0 a	5.0 a	4.9 a	4.0 a	3.0 a
NuCOTN 33B	4.8 a	4.0 b	3.8 b	3.5 a	2.4 a

*Means followed by the same letter within a column for a given species do not significantly differ (P=0.05, Duncan's MRT).

Table 3. Cumulative percent leaf damage by beet armyworm larvae as affected by Dimilin and Bt transgenic cotton (NuCOTN 33B) across 5 days of bioassay (DOB). Evaluation 1.

	Dimilin	Cumulative % leaf damage				
Treatment	(ppm)	1 DOB	2 DOB	3 DOB	4 DOB	5 DOB
Stonv 453	0	9 a*	28 a	75 a	111 a	138 a
Stonv 453	47	8 a	20 b	27 bc	34 c	45 bc
NuCOTN33B	0	7 a	19 b	40 b	58 b	73 b
NuCOTN33B	47	7 a	13 b	16 c	18 c	19 c
Stonv 453	0	9 a	28 a	75 a	111 a	138 a
Stonv 453	12	9 a	30 a	73 a	106 a	120 a
NuCOTN33B	0	7 ab	19 b	40 b	58 b	73 b
NuCOTN33B	12	6 b	12 b	19 c	24 c	26 c
Stonv 453	0	9 ab	28 a	75 a	111 a	138 a
Stonv 453	3	10 a	27 a	65 a	88 a	98 b
NuCOTN33B	0	7 b	19 ab	40 b	58 b	73 b
NuCOTN33B	3	7 b	17 b	23 b	30 c	34 c

*Means followed by the same letter within a column for a given concentration of Dimilin do not significantly differ (P=0.05, Duncan's MRT).

Table 4. Survival of beet armyworm as affected by Dimilin and transgenic Bt cotton (NuCOTN 33B) across 5 days of bioassay (DOB). Evaluation 1.

Treatment	Dimilin (ppm)	Mean # of Larvae Surviving / 5 1 DOB	2 DOB	3 DOB	4 DOB	5 DOB
Stonv 453	0	5.0 a*	5.0 a	4.9 a	4.6 a	4.1 a
Stonv 453	47	4.3 b	4.0 a	3.3 b	1.6 b	0.5 c
NuCOTN33B	0	5.0 a	4.8 a	4.5 a	3.6 a	2.8 b
NuCOTN33B	47	4.5 ab	3.8 a	2.1 c	0.6 b	0.1 c
Stonv 453	0	5.0 a	5.0 a	4.9 a	4.6 a	4.1 a
Stonv 453	12	4.8 a	4.6 a	4.5 a	3.9 a	2.4 bc
NuCOTN33B	0	5.0 a	4.8 a	4.5 a	3.6 a	2.8 b
NuCOTN33B	12	4.6 a	4.5 a	3.8 a	1.9 b	1.3 c
Stonv 453	0	5.0 a	5.0 a	4.9 a	4.6 a	4.1 a
Stonv 453	3	5.0 a	4.9 a	4.6 a	3.6 ab	1.6 bc
NuCOTN33B	0	5.0 a	4.8 a	4.5 a	3.6 ab	2.8 b
NuCOTN33B	3	4.6 b	4.5 a	4.1 a	2.4 b	0.9 c

*Means followed by the same letter within a column for a given concentration of Dimilin do not significantly differ (P=0.05, Duncan's MRT).

Table 5. Cumulative percent leaf damage by beet armyworm larvae as affected by Dimilin and Bt transgenic cotton (NuCOTN 33B) across 5 days of bioassay (DOB). Evaluation 2.

Treatment	Dimilin (ppm)	1 DOB	Cumulative % leaf damage 2 DOB	3 DOB	4 DOB	5 DOB
Stonv 453	0	12 a*	36 a	84 a	122a	163 a
Stonv 453	47	12 a	22 b	49 b	58 b	69 b
NuCOTN33B	0	8 b	18 b	48 b	65 b	78 b
NuCOTN33B	47	7 b	18 b	35 b	39 b	42 b
Stonv 453	0	12 a	36 a	84 a	122a	163 a
Stonv 453	12	11 a	25ab	59 b	74 b	97 b
NuCOTN33B	0	8 a	18 b	48bc	65 b	78 bc
NuCOTN33B	12	7 b	15 b	25 c	29 c	37 c
Stonv 453	0	12ab	36 a	84 a	122a	163 a
Stonv 453	3	12 a	44 a	88 a	140a	143 a
NuCOTN33B	0	8 c	18 b	48 b	65 b	78 b
NuCOTN33B	3	9 bc	18 b	50 b	61 b	72 b

*Means followed by the same letter within a column for a given concentration of Dimilin do not significantly differ (P=0.05, Duncan's MRT).

Table 6. Survival of beet armyworm as affected by Dimilin and transgenic Bt cotton (NuCOTN 33B) across 5 days of bioassay (DOB). Evaluation 2.

Treatment	Dimilin (ppm)	1 DOB	Cumulative % leaf damage 2 DOB	3 DOB	4 DOB	5 DOB
Stonv 453	0	4.6 a*	4.1 a	3.6 ab	3.4 a	3.0 a
Stonv 453	47	4.6 a	3.5 a	2.5 bc	1.6 b	0.6 b
NuCOTN33B	0	4.6 a	4.4 a	3.9 a	3.8 a	3.3 a
NuCOTN33B	47	4.4 a	3.6 a	2.0 c	1.4 b	0.9 b
Stonv 453	0	4.6 a	4.1 a	3.6 a	3.4 a	3.0 a
Stonv 453	12	4.9 a	4.6 a	3.8 a	2.5 ab	1.8 ab
NuCOTN33B	0	4.6 a	4.4 a	3.9 a	3.8 a	3.3 a
NuCOTN33B	12	4.3a	3.6 a	3.3a	2.0 b	0.8 b
Stonv 453	0	4.6 a	4.1 a	3.6 a	3.4 a	3.0 a
Stonv 453	3	4.8 a	4.5 a	4.1 a	3.9 a	2.5 a
NuCOTN33B	0	4.6 a	4.4 a	3.9 a	3.8 a	3.3 a
NuCOTN33B	3	4.9 a	4.4 a	3.9 a	3.1 a	2.0 a

*Means followed by the same letter within a column for a given concentration of Dimilin do not significantly differ (P=0.05, Duncan's MRT).

Table 7. Cumulative percent leaf damage by fall armyworm larvae as affected by Dimilin and Bt transgenic cotton NuCOTN 33B) across 5 days of bioassay (DOB).

Treatment	Dimilin (ppm)	1 DOB	Cumulative % leaf damage 2 DOB	3 DOB	4 DOB	5 DOB
Stonv 453	0	13 a*	18 a	22 a	38 a	52 a
Stonv 453	47	11 ab	16 ab	19 a	20 b	20 c
NuCOTN33B	0	8 bc	14 bc	18 a	22 b	36 b
NuCOTN33B	47	7 c	11 c	16 a	19 b	22 c
Stonv 453	0	13 a	18 a	22 a	38 a	52 a
Stonv 453	12	11 ab	15 a	17 ab	19 b	24 b
NuCOTN33B	0	8 c	14 a	18 ab	22 b	36 b
NuCOTN33B	12	9 bc	13 a	14 b	19 b	26 b

*Means followed by the same letter within a column for a given concentration of Dimilin do not significantly differ (P=0.05, Duncan's MRT).

Table 8. Survival of fall armyworm as affected by Dimilin and transgenic Bt cotton (NuCOTN 33B) across 5 days of bioassay (DOB).

Treatment	Dimilin (ppm)	1 DOB	Cumulative % leaf damage 2 DOB	3 DOB	4 DOB	5 DOB
Stonv 453	0	4.4 a*	4.3 a	3.9 a	3.4 a	2.5 a
Stonv 453	47	4.0 a	3.1 a	1.4 c	0.3 d	0.0 b
NuCOTN33B	0	3.5 a	3.3 a	3.1 ab	2.5 b	1.8 a
NuCOTN33B	47	4.1 a	3.9 a	2.4 bc	1.1 c	0.4 b
Stonv 453	0	4.4 a	4.3 a	3.9 a	3.4 a	2.5 a
Stonv 453	12	4.4 a	3.5 a	2.3 a	1.3 b	0.8 b
NuCOTN33B	0	3.5 a	3.3 a	3.1 a	2.5 a	1.8 ab
NuCOTN33B	12	4.4 a	3.9 a	3.0 a	2.8 a	1.5 ab

*Means followed by the same letter within a column for a given concentration of Dimilin do not significantly differ (P=0.05, Duncan's MRT).

Table 9. Cumulative percent leaf damage by soybean looper larvae as affected by Dimilin and Bt transgenic cotton (NuCOTN 33B) across 5 days of bioassay (DOB).

Treatment	Dimilin (ppm)	1 DOB	2 DOB	3 DOB	4 DOB	5 DOB
Stonv 453	0	11 a*	26 a	84 a	114a	166 a
Stonv 453	748	9 a	19 a	34 b	37 b	41 b
NuCOTN33B	0	3 b	5 b	5 c	5 c	5 c
NuCOTN33B	748	3 b	3 c	3 c	4 c	4 c
Stonv 453	0	11 b	26 a	84 a	114a	166 a
Stonv 453	187	14 a	28 a	64 b	75 b	94 b
NuCOTN33B	0	3 c	5 b	5 c	5 c	5 c
NuCOTN33B	187	3 c	4 b	4 c	4 c	4 c

*Means followed by the same letter within a column for a given concentration of Dimilin do not significantly differ (P=0.05, Duncan's MRT).

Table 10. Survival of soybean looper as affected by Dimilin and transgenic Bt cotton (NuCOTN 33B) across 5 days of bioassay (DOB).

Treatment	Dimilin (ppm)	Cumulative % leaf damage 1 DOB	2 DOB	3 DOB	4 DOB	5 DOB
Stonv 453	0	4.6a*	4.5 a	4.5 a	4.3 a	4.0 a
Stonv 453	748	4.1 a	3.3 b	2.0 b	1.6 b	0.4 b
NuCOTN33B	0	4.6 a	1.5 c	0.1 c	0.1 c	0.1 b
NuCOTN33B	748	4.5 a	2.1 c	0.1 c	0.1 c	0.0 b
Stonv 453	0	4.6 a	4.5 a	4.5 a	4.3 a	4.0 a
Stonv 453	187	4.6 a	4.3 a	3.6 b	3.3 b	2.1 b
NuCOTN33B	0	4.6 a	1.5 b	0.1 c	0.1 c	0.1 c
NuCOTN33B	187	4.9 a	2.0 b	0.1 c	0.0 c	0.0 c

*Means followed by the same letter within a column for a given concentration of Dimilin do not significantly differ (P=0.05, Duncan's MRT).

Table 11. Cumulative percent leaf damage by cotton bollworm larvae as affected by Dimilin and Bt transgenic cotton (NuCOTN 33B) across 5 days of bioassay (DOB).

Treatment	Dimilin (ppm)	Cumulative % leaf damage 1 DOB	2 DOB	3 DOB	4 DOB	5 DOB
Stonv 453	0	7b**	11 a	20 a		27 a
Stonv 453	187	10 a	12 a	15 a		25 a
NuCOTN33B	0	1 c	1 b	2 b		2 b
NuCOTN33B	187	1 c	1 b	2 b		2 b
Stonv 453	0	7 b	11 a	20 a		27 a
Stonv 453	47	10 a	13 a	18 a		32 a
NuCOTN33B	0	1 c	1 b	2 b		2 b
NuCOTN33B	47	1 c	1 b	1 b		1 b

*Not determined

**Means followed by the same letter within a column for a given concentration of Dimilin do not significantly differ (P=0.05, Duncan's MRT).

Table 12. Survival of cotton bollworm as affected by Dimilin and transgenic Bt cotton (NuCOTN 33B) across 5 days of bioassay (DOB).

Treatment	Dimilin (ppm)	Cumulative % leaf damage 1 DOB	2 DOB	3 DOB	4 DOB	5 DOB
Stonv 453	0	3.9 a**	2.9 a	1.9 a		1.3 a
Stonv 453	187	4.4 a	3.0 a	1.4 a		0.5 b
NuCOTN33B	0	4.0 a	1.3 b	0.1 b		0.0 b
NuCOTN33B	187	3.6 a	1.1 b	0.1 b		0.0 b
Stonv 453	0	3.9 a	2.9 a	1.9 a		1.3 a
Stonv 453	47	4.4 a	2.9 a	1.4 a		0.6 b
NuCOTN33B	0	4.0 a	1.3 b	0.1 b		0.0 b
NuCOTN33B	47	3.9 a	1.6ab	0.0 b		0.0 b

*Not determined

**Means followed by the same letter within a column for a given concentration of Dimilin do not significantly differ (P=0.05, Duncan's MRT).