COMPARISON OF DIFFERENT CHEMISTRIES AND RATES AGAINST BOLLWORM IN BT AND CONVENTIONAL COTTON

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Abstract

Six insecticides of different chemistries were evaluated against the cotton bollworm (*Helicoverpa zea* [Boddie]) in conventional (Deltapine® 'DP 5415', Deltapine® 'DP 5415RR') and transgenic *Bacillus thuringiensis* (Berliner) (Deltapine® 'NuCOTN 33B', Deltapine® 'DP 458 B/RR') cotton. In 1998, treatments consisting of three rates each of a pyrethroid (Karate-Z®), spinosyn (Tracer®), carbamate (Larvin®), pyrrole (Pirate®), oxadiazine (Steward®), and avermectin (Proclaim®) were established in a non-irrigated field. In 1999, treatments consisted of three rates each of Karate-Z®, Tracer®, Larvin®, and Steward® in an irrigated and a non-irrigated (dryland) field. The highest rate of each insecticide corresponded to normal grower-use rates. Tracer® and Larvin® controlled *H. zea* in conventional cotton, whereas other materials were less effective. Even though *H. zea* is becoming increasingly resistant to pyrethroid insecticides, Karate-Z® was highly effective in dryland *B. thuringiensis* (B.t.) cotton. Tracer® and Larvin® were equally effective. Data indicated that reduced rates of Karate-Z®, Tracer® and Larvin® could be used for control of *H. zea* in dryland B.t. cotton systems. However, reduced rates of these insecticides in an irrigated B.t. cotton system did not provide adequate control.

Introduction

The cotton bollworm (*Helicoverpa zea* [Boddie]) is becoming more tolerant to pyrethroid insecticides (Brown et al. 1997, Roof et al. 1998). While alternative control strategies typically rely on transgenic cotton expressing CryIA(c) endotoxins of *Bacillus thuringiensis* subsp. *kurstaki* (Berliner), most growers are forced to apply one to three supplemental insecticide applications to their B.t. cotton for bollworm control. Thus the need for information on pyrethroid and alternative chemistries for *H. zea* control in transgenic B.t. cotton.

Field and laboratory observations have shown that larvae of *H. zea* that survive on transgenic B.t. cotton are smaller and develop slower than those in conventional cotton (Meyers 1997, Sims et al. 1996). Harris et al. (1998) found that larvae of *H. zea* exposed to sublethal doses of B.t. toxins were more susceptible to the pyrethroid cyhalothrin, than those not exposed to the toxins. Additional studies are needed to determine whether the physiological state of surviving larvae of *H. zea* may result in a synergism between the B.t. toxin and other insecticides. If such a synergism is demonstrated, it might be possible to supplement control of *H. zea* in B.t. cotton, using lower rates of insecticides. Use of reduced rates would lower insecticide costs and result in higher populations of beneficial arthropods which provide additional insect control. However, reduced rates might not provide adequate control of secondary pests such as stink bugs (*Acrosternum hilare* [Say], *Nezara viridula* [L.] and *Euschistus servus* [Say]), which are not affected by B.t. toxins.

We assessed control of *H. zea* in conventional and B.t. cotton, following applications of various rates of an insecticide from classes of chemistry with different modes of action. Classes of chemistry and insecticides were pyrethroid (Karate-Z®), spinosyn (Tracer®), carbamate (Larvin®), oxidiazine (Steward®), pyrrole (Pirate®) and avermectin (Proclaim®). This rate study should allow us to determine whether or not a synergistic relationship exists between B.t. cotton and any of these insecticides.

Materials and Methods

1998 Studies

A field study was conducted near Denmark, South Carolina, in adjacent plantings of conventional (Deltapine® 'DP 5415') and B.t. (Deltapine® 'NuCOTN 33B') cotton in a non-irrigated (dryland) field on the Sandifer farm. Treatments consisted of a check (untreated for *H. zea*) and three rates each of Karate-Z®, Tracer®, Larvin®, Steward®, Pirate®, and Proclaim®. The highest rate for each insecticide generally corresponded to normal grower-use rates. This rate was reduced by either 1/2 and 1/4 or 2/3 and 1/3, for a total of three rates per insecticide (Table 1). Cotton was planted on 12 May. Five replicates of plots, eight rows wide (7.16 m) by 12.2 m long, were established in a randomized complete block. Acephate (0.56 kg (AI)/ha) was applied on 25 June and 6 July to reduce beneficial arthropods and thereby increase *H. zea* larval populations later in July (Turnipseed and Sullivan 1999). The treatment threshold for *H. zea* on B.t. cotton in South Carolina is based on either 75 eggs, 30 small larvae (< 2/3 cm in length) or three large larvae (2/3 cm or longer) per 100 plants (Sullivan et al. 1998). The initial flight of *H. zea* from corn into cotton was especially high in 1998 and the threshold of 75 eggs/100 plants was used for both genotypes. Initial treatments were applied on 9 July 1998, using a CO^2 backpack sprayer, with 5X hollow cone nozzles that delivered 88.9 liters/ hectare. Subsequent applications were made weekly for a total of four within both genotypes. Treatment effects were monitored using a 1-meter square beat cloth (Shepard et al. 1974). Three samples were made in each plot by placing the cloth between rows, bending plants from one

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 2:971-975 (2000)
National Cotton Council, Memphis TN

row over and beating them downward to dislodge larvae onto the cloth. Sampling dates were 21 July (3 days after the second application), 27 July (4 days after the third application), 3 August (4 days after the fourth application) and 10 August (11 days after the fourth application). Only *H. zea* larvae 2/3 cm or longer were recorded. Treatment effects were analyzed using analysis of variance (ANOVA) (PROC GLM, SAS Institute 1988). Data were transformed as follows: $\sqrt{(y + 0.5)}$ and means were separated using the Fisher protected least significant difference (LSD) test for all data.

The impact of B.t. cotton on the weight of *H. zea* larvae was assessed by taking random beat cloth samples 21 days after initial egg deposition within plots of conventional and B.t. genotypes that were not treated for *H. zea*. Approximately 60 larvae were collected, placed individually in plastic cups, taken to the laboratory and weighed.

1999 Studies

In adjacent plantings of conventional and B.t. cotton an untreated check and three rates each of Karate-Z®, Tracer®, Larvin® and Steward® were compared in a dryland field on the Edisto Research and Education Center (EREC) near Blackville, S.C., and in an irrigated field on the Bamberg farm near Bamberg, S.C. Rates were the same as those used in 1998. In the EREC field, conventional ('DPL 5415') and B.t. ('NuCOTN 33B') varieties were planted on 10 May 1999. In the Bamberg field, transgenic varieties expressing the Roundup Ready™ (RR) gene (Deltapine® 'DP 5415RR') and the Roundup Ready™ gene in combination with the Bollgard™ (BG) gene (Deltapine® 'DP 458 B/RR'), were planted on 8 May. Five replicates of plots, ten rows wide (9.7 m) by 12.2 m long, were established in a randomized complete block. Acephate was applied (0.56 kg (AI)/ha) to each field on 2 and 10 July. The initial flight of *H. zea* from corn into cotton was less intense and occurred later than in 1998. Thresholds for treatment of *H. zea* in conventional cotton, three large larvae (> 2/3 cm in length) per 100 plants and in B.t. cotton were 30 small larvae (< 2/3 cm in length) per 100 plants. Initial applications to conventional plots were made on 20 July (EREC) and 21 July (Bamberg). Initial applications to B.t. plots were made on 23 July (EREC and Bamberg). Application methods, equipment and spray volumes were similar to those used in 1998. Subsequent applications to both genotypes were made on the above-described thresholds rather than on automatic weekly applications as in 1998. Total applications consisted of three to conventional and two to B.t. plots. Sampling methods were similar to those used in 1998. Beat cloth samples were taken in the EREC field as follows: in the conventional plots on 30 July (3 days after the second application) and 4 August (4 days after the third application) and in the B.t. plots on 2 August (4 days after the second application) and 6 August (8 days after the second application). Similar samples were taken at the Bamberg field as follows: in the conventional plots on 30 July (4 days after the second application) and 4 August (4 days after the third application) and in the B.t. plots on 3 August (5 days after the second application) and 6 August (8 days after the second application). Treatment effects were analyzed using analysis of variance (ANOVA) (PROC GLM, SAS Institute 1988). Data were transformed as follows: $\sqrt{(y + 0.5)}$ and means were separated using the Fisher protected least significant difference (LSD) test for all data. Collections of *H. zea* larvae were made in both fields and genotypes at 6 and 19 days after the initial flight in order to determine weight differences. Procedures and analyses were similar to those used in 1998.

Results

1998 Studies

Cumulative totals of *H. zea* larvae for 21 and 27 July, expressed as mean no. per 100 plants, are presented in Table 1. In conventional cotton, the highest rate of Karate-Z® (0.028 kg [AI] / ha) was significantly less effective than the highest rates of Tracer® (0.101 kg [AI] / ha) (69% vs. 92% control, respectively [Table 1]). Larvin® (0.897 kg [AI] / ha) and Steward® (0.101 kg [AI] / ha) provided adequate control in conventional cotton (87% and 88% control, respectively [Table 1]). Pirate® and Proclaim® were both ineffective against *H. zea* in conventional cotton. In B.t. cotton, there were no significant differences among the highest rates of Karate-Z®, Tracer® and Larvin® (100%, 97%, 97% control respectively [Table 1]). At all rates, the efficacy of Karate-Z® increased in B.t. vs. conventional cotton but those of Tracer® did not increase in B.t. cotton (Table 1). Data in Fig. 1 include the mean number of larvae per 100 plants for conventional plots treated with the highest rate of Karate-Z®, Tracer®, Larvin® and Steward®; for B.t. plots treated with the middle rates of these insecticides; and for untreated B.t. plots. In B.t. cotton, the middle rates of Karate-Z® (0.015 kg [AI] / ha), Tracer® (0.067 kg [AI] / ha), Larvin® (0.448 kg [AI] / ha) and Steward® (0.05 kg [AI] / ha) consistently maintained larval counts at or below five per 100 plants. *Helicoverpa zea* larvae collected from conventional cotton 21 days after the initial flight were significantly larger than those collected from B.t. cotton (171.0 mg vs. 98.2 mg [F = 2.08; df = 59, 61; P < 0.001], respectively).

1999 Studies

Cumulative totals of *H. zea* larvae for three separate sampling dates, in each genotype, expressed as mean no. per 100 plants, are presented in Tables 2 and 3. In dryland conventional cotton, there were no significant differences among the highest rates of Karate-Z®, Tracer® or Larvin® (86%, 90% and 93% control, respectively [Table 2]); however, the highest rate of Larvin® provided significantly greater control than the highest rate of Steward®. As in 1998, Karate-Z® provided a higher percent control in B.t. than in conventional cotton, whereas the efficacy of Tracer® at middle and low rates decreased in B.t. cotton (Table 2). In B.t. cotton, the middle rates of Karate-Z® (0.015 kg [AI] / ha), Tracer® (0.067 kg [AI] / ha) and Larvin® (0.448 kg [AI] / ha) consistently kept larval counts below five per 100 plants (Fig. 1, dryland 1999).

In the irrigated field (Bamberg), there were no significant differences among the highest rates of Karate-Z®, Tracer® and Larvin® in either the conventional (93, 93, and 95%, respectively) or B.t. (87, 94 and 92%, respectively) genotypes. All rates of Karate-Z® provided a lower percent control in B.t. compared with conventional cotton. Although there were little differences at the highest rate, the percent control afforded by Tracer® at the middle and lowest rate decreased (Table 3). In B.t. cotton the middle rates of Karate-Z®, Tracer, Larvin® and Steward® did not keep larval counts below five per 100 plants at five and nine days after the second application (Fig. 1, irrigated 1999).

In the dryland field (EREC), larvae of *H. zea* collected from conventional cotton were significantly larger than those collected from B.t. cotton at 6 and 19 days following the initial bollworm flight (75.1 mg vs. 6.0 mg [$F = 19.02$; df = 29, 28; $P < 0.001$] and 129.8 mg vs. 261.0 mg [$F = 4.28$; df = 29, 28; $P < 0.001$], respectively). Similar weight variations between conventional and B.t. cotton were observed in larvae collected from the irrigated field (Bamberg) at 6 days following the flight (87.4 mg vs. 4.6 mg [$F = 109.14$; df = 29, 25; $P < 0.001$], for larvae from conventional and B.t. cotton, respectively); however, at 19 days there were no significant differences between larvae from the conventional and B.t. genotypes (217.2 mg vs. 188.8 mg [$F = 1.17$; df = 29, 29; $P < 0.001$], for larvae from conventional and B.t. cotton, respectively).

Discussion

Turnipseed and Sullivan (1999) and Hagerty et al. (2000) reported that the destruction of beneficial arthropods following applications of acephate resulted in unusually high populations of larvae of *H. zea*. We made similar applications to each field to enhance larval numbers. Survival of larvae of *H. zea* in untreated B.t. plots ranged from 15 to 42 large larvae per 100 plants (Tables 1,2 and 3). Greenplate et al. (1998) attributed this survival to the following: 1. variations in CryIA(c) susceptibility among *H. zea* populations; 2. the ability of larvae to avoid high concentrations of CryIA(c) during early instars by feeding within blooms where expression of the toxin is low; and 3. a reduction in CryIA(c) expression by the plant due to environmental and physiological conditions. In B.t. cotton, we consistently observed second and third-instar larvae of *H. zea* feeding in fresh blooms and on small bolls under bloom tags (i.e. 23 larvae per 100 blooms on 28 July, within the dryland field in 1999). Halcomb et al. (1996) reported that larvae of *H. zea* fed transgenic B.t. flower buds weighed significantly less than those fed non-transgenic flower buds. Our results were similar, in that larvae of *H. zea* within B.t. plots, under dryland conditions, weighed significantly less and generally exhibited slower development than those within conventional plots. Under irrigated conditions, larvae collected from B.t. plots 6 days after the flight were significantly smaller than those collected from conventional ones; however, at 19 days there were no significant differences in larval size. This may have been due to high humidity that enhanced larval growth within the irrigated foliage and/or excessive vegetative growth that reduced the amount of endotoxin expressed by the B.t. plants (Benedict et al. 1996). Also, it is likely that at 19 days following the initial flight, larger larvae within the conventional genotype had pupated (Pfadt 1962), allowing slower-developing larvae in the B.t. genotype to attain similar weights.

Pyrethroid insecticides historically have provided excellent control of many agricultural pests. However, widespread use of these insecticides has resulted in increased tolerance among *H. zea* populations. Wang et al. (1994) and Tan et al. (1998) found that sublethal exposure of the bollworm, *Helicoverpa armigera* (Hubner), to a *B. thuringiensis* insecticide resulted in a decrease in pyrethroid resistance. In our 1998 studies, Karate-Z® did not provide adequate control of *H. zea* in dryland conventional cotton whereas Tracer® and Larvin® provided good control. However, in B.t. cotton all rates of Karate-Z®, Tracer®, Larvin® and Steward® generally produced similar control. In both 1998 and 1999 dryland B.t. plots, intermediate rates of Karate-Z®, Tracer® and Larvin® controlled *H. zea* populations. The highest rate of Karate-Z®, a pyrethroid insecticide that acts primarily as a contact poison (Sparks et al. 1995), demonstrated a substantial increase in efficacy when applied to dryland B.t. cotton (69% in conventional vs. 100% in B.t., Table 1; 86% in conventional vs. 95% in B.t., Table 2). The highest rate of Larvin®, a carbamate insecticide that acts both as a contact and stomach poison, demonstrated a moderate increase in efficacy when applied to dryland B.t. cotton (87% vs. 97%, Table 1; 92% vs. 96%, Table 2). However, Tracer® and Steward® which require ingestion to be most effective (Sparks et al. 1995, DuPont 1997), demonstrated little or no increase in percent control in B.t. cotton. LC50 values determined for field collected larvae (Brickle et al. 1999) were consistent with this pattern, in that λ-cyhalothrin was the only insecticide with a lower LC50 value for larvae collected from B.t. cotton. These observations suggest that insecticides, which are contact poisons, might be more effective in B.t. than conventional cotton. However, disruption of the gut by B.t. endotoxins might negate this increase in efficacy for insecticides that require ingestion. More detailed studies are needed to determine if mode of entry influences the efficacy associated with these chemistries and B.t. cotton. Our study also suggests that exposure to transgenic B.t. cotton can help mediate resistance of *H. zea* to pyrethroids and thereby increase their efficacy and perhaps longevity.

Irrigated B.t. cotton ('DP 458 B/RR') was less effective against *H. zea* than dryland B.t. cotton ('NuCOTN 33B'). This might be attributed to a reduction in endotoxin expression due to excessive soil moisture and vegetative growth (Benedict et al. 1996); however, endotoxin expression might be intrinsically different between the two genotypes. Unlike dryland fields (Tables 1 and 2), treatments to the irrigated field (Table 3) were generally less effective on roundup ready / B.t. ('DP 458 B/RR') than on roundup ready / conventional ('DP 5415RR') cotton. Since applications in 1999 were made to the two genotypes on different dates, varying degrees of moisture and/or endotoxin expression may have reduced the synergistic effect that was observed between Karate-Z® and B.t. in dryland fields. Further examination is needed to clarify the suggested interaction of water stress and endotoxin expression.

Data from this study suggest that lower rates of Karate-Z®, Tracer® and Larvin® can be used to control *H. zea* populations in dryland B.t. cotton. However, lower rates under irrigated conditions were not effective and should not be recommended.

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Table 1. Efficacy of different insecticides and rates against *H. zea* in conventional (‘DP 5415') and B.t. ('NuCOTN 33B') cotton at the dryland Sandifer field, 1998.

		Mean larvae / 100 plants[a] ± SE		Percent control	
Chemical	**Rate kg. [AI] / ha**	**‘DP 5415’[b]**	**‘NuCOTN 33B’[c]**	**‘DP 5415’**	**‘NuCOTN 33B’**
Karate-Z®	0.028	16.0±10.2d-g	0.0±0.0d	68.5	100.0
Karate-Z®	0.015	17.6±5.5c-e	3.1±3.1bcd	65.3	79.4
Karate-Z®	0.007	28.6±18.5bc	3.3±3.6bcd	43.7	77.8
Tracer®	0.101	4.1±3.1h	0.5±1.1d	92.0	96.8
Tracer®	0.067	8.3±5.5f-h	3.1±2.3bcd	83.6	79.4
Tracer®	0.034	12.4±7.0e-h	5.2±3.9bc	75.6	65.1
Larvin®	0.897	6.7± 5.1gh	0.5±1.1d	86.9	96.8
Larvin®	0.448	12.9±1.8e-h	2.4±3.4cd	74.7	84.1
Larvin®	0.224	17.4±7.1c-f	3.3±3.6cd	65.7	77.8
Steward®	0.101	6.2±5.5gh	2.4±3.4cd	87.8	84.1
Steward®	0.050	8.6±7.2gh	4.1± 2.7bc	83.1	73.0
Steward®	0.025	12.1±5.4e-h	2.4± 2.9cd	76.1	84.1
Pirate®	0.336	25.0±17.1b-d	6.2± 6.9bc	50.7	58.7
Pirate®	0.224	27.6±16.9b-d	7.9± 5.4b	45.5	47.6
Pirate®	0.112	38.8±20.4ab	6.4± 6.3bc	23.5	57.1
Proclaim®	0.014	11.2±5.0e-h	3.1± 3.5bcd	77.9	79.4
Proclaim®	0.007	18.8±9.5c-e	7.4± 5.5b	62.9	50.8
Proclaim®	0.004	28.3±9.3b-d	4.8± 3.0bc	44.1	68.3
Untreated	0.0	50.7±23.0a	15.0± 7.5a		

Means ± SE, means with different letter are significantly different at $\alpha = 0.05$.

[a] Mean derived from total larvae collected on two sampling dates (21 July [after the second application] and 27 July [after the third application]) with counts corrected to give no. larvae / 100 plants.

[b] LSD means separation test (F=6.24 ; df=94; P ≤ 0.0001).

[c] LSD means separation test (F=4.04 ; df=94; P ≤ 0.0001).

Table 2. Efficacy of different insecticides and rates against *H. zea* in conventional ('DP 5415') and B.t. ('NuCOTN 33B') cotton at the dryland EREC field, 1999.

Chemical	Rate kg [AI] / ha	Mean larvae / 100 plants[a] ± SE		Percent Control	
		'DP 5415'[b]	'NuCOTN 33B'[c]	'DP 5415'	'NuCOTN 33B'
Karate-Z®	0.028	19.0±11.0gh	1.4±1.3d	86.4	94.6
Karate-Z®	0.015	39.4±15.8c-e	3.8±4.3cd	71.9	85.7
Karate-Z®	0.007	68.4±19.7b	6.7±2.0bc	51.2	75.0
Tracer®	0.101	14.7±9.0gh	3.8±3.6cd	89.5	85.7
Tracer®	0.067	19.5±9.6f-h	4.3±5.2b-d	86.1	83.9
Tracer®	0.034	31.4±17.1d-g	7.1±5.8bc	77.6	73.2
Larvin®	0.897	10.0±8.5h	1.0±1.3d	92.9	96.4
Larvin®	0.448	18.1±13.3gh	3.8±4.3cd	87.1	85.7
Larvin®	0.224	36.6±6.4c-f	5.7±4.6b-d	73.9	78.6
Steward®	0.101	24.2±5.4e-g	3.8±2.7cd	82.7	85.7
Steward®	0.050	53.7±19.3b-d	7.6±5.9bc	61.7	71.4
Steward®	0.025	55.1±26.8bc	10.0±6.8b	60.7	62.5
Untreated	0.0	140.1±49.6a	26.6±14.1a		

Means ± SE, means with different letter are significantly different at $\alpha = 0.05$.

[a] Mean derived from total larvae collected on two sampling dates (30 July [after second application] and 4 August [after third application] in 'DP 5415'; 2 August [after second application] and 6 August [after second application] in 'NuCOTN 33B'; with counts corrected to give no. larvae per 100 plants.

[b] LSD means separation test ($F=10.44$; $df=64$; $P \leq 0.0001$).

[c] LSD means separation test ($F=5.29$; $df=64$; $P \leq 0.0001$).

Table 3. Efficacy of different insecticides and rates against *H. zea* following three applications in conventional ('DP 5415RR') cotton and two applications in B.t. ('DP 458 B/RR') cotton in the irrigated Bamberg field, 1999.

Chemical	Rate kg [AI] / ha	Mean larvae / 100 plants[a] ± SE		Percent Control	
		'DP 5415RR'[b]	'DP 458 B/RR'[c]	'DP 5415RR'	'DP 458 B/RR'
Karate-Z®	0.028	8.9±5.3gh	5.4±7.6e	93.1	87.1
Karate-Z®	0.015	22.0±9.6d-f	17.3±6.8bc	83.0	58.6
Karate-Z®	0.007	74.4±27.3b	18.4±13.5bc	42.7	55.7
Tracer®	0.101	8.3±4.1gh	2.4±3.4e	93.6	94.3
Tracer®	0.067	13.7±3.0e-h	12.5±2.3b-d	89.5	70.0
Tracer®	0.034	20.2±8.1d-g	11.9±6.1b-d	84.4	71.4
Larvin®	0.897	6.5±7.1h	3.0±3.6e	95.0	92.9
Larvin®	0.448	17.9±4.6d-g	13.7±4.9bc	86.2	67.1
Larvin®	0.224	26.2±10.1de	17.3±11.4bc	79.8	58.6
Steward®	0.101	11.3±7.9f-h	7.1±0.0c-e	91.3	82.9
Steward®	0.050	33.9±16.7cd	13.7±7.9b-d	73.9	67.1
Steward®	0.025	51.8±20.0b	22.0±15.4b	60.1	47.1
Untreated	0.0	129.8±33.4a	41.7±10.0a		

Means ± SE, means with different letter are significantly different at $\alpha = 0.05$.

[a]Mean derived from total larvae collected on two sampling dates (30 July [after second application] and 4 August [after third application] in 'DP 5415RR'; 3 August [after second application] and 6 August [after second application] in DP 458 B/RR') with counts corrected to give no. larvae per 100 plants.

[b] LSD means separation test ($F=16.43$; $df=51$; $P \leq 0.0001$).

[c] LSD means separation test ($F=4.58$; $df=51$; $P \leq 0.0001$).

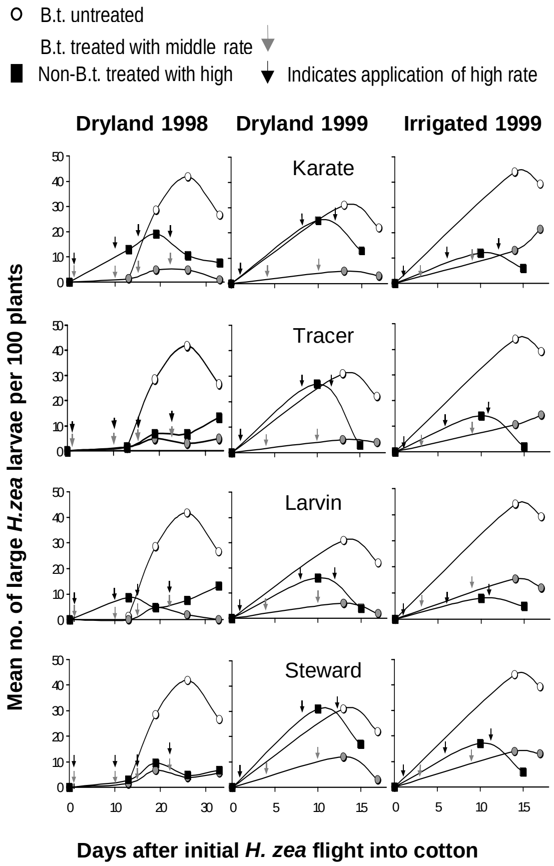

Figure 1. Larvae of *H. zea* sampled by beat cloth within B.t. plots left untreated, B.t. plots treated with the middle rate and conventional plots treated with the highest rate of insecticides.