GROWTH AND DEVELOPMENT OF NONRESISTANT AND BT-RESISTANT SOYBEAN LOOPERS ON FOLIAGE FROM NONBT- AND BT-COTTON

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Abstract

Survival and growth of Bt-resistant and nonresistant soybean loopers on leaves collected from the upper, middle and lower canopy of Bt- and nonBt-cotton plants were studied. Survival of Bt-resistant and nonresistant larvae after 10 days was not affected by the cotton variety. However, nonresistant larvae fed leaves from nonBt-cotton had significantly higher survival through pupation and adult emergence than those fed Bt-cotton. Survival of larvae from both cultures through pupation and adult emergence was significantly higher when fed leaves from nodes in the middle than from upper or lower canopy of both varieties of cotton. Nonresistant larvae fed leaves from nonBt-cotton had a significantly greater larval weight and shorter development period than those fed Bt-cotton.

Introduction

The soybean looper is a major pest of soybean (Felland et al. 1992). It has also appeared as an important pest of cotton (Sprenkel and Johnson 1998). Transgenic cotton that expressed an endotoxin protein (Cry1Ac) of *B. thuringiensis* (Bt-cotton) was introduced as an alternative to traditional insecticides against lepidopterous pests of cotton, and significantly reduced larval survival and weight of bollworm and tobacco budworm (Jenkins et al. 1997; Adamczyk et al. 1998; Parker et al. 2000). In addition, a reduction in the pupal weight and an extension in the pupal developmental period in the beet armyworm (Stapel et al. 1997), and larval survival to pupation and adult eclosion in the fall armyworm (Adamczyk et al. 1998) were also reported. A reduction in survivability and growth of nonresistant soybean looper on Bt-cotton has also been observed (M. Ashfaq and S. Young, unpublished data). The objective of this study was to determine the effect of leaf location (upper, middle and lower nodes) of nonBt and Bt-cotton on survival and growth of Bt-resistant and nonresistant strains of the soybean looper.

Materials and Methods

Plants and Insects

To determine the affect of leaf location, lower (nodes 1 to 4), middle (nodes 6 to 8) and upper (nodes 9 to 12) leaves were collected from pre-bloom cotton, *Gossypium hirsutum* L., var. Deltapine 51 (nonBt-cotton) and var. DP 428 B (Bt-cotton) grown on research plots at the Agricultural Experiment Station, University of Arkansas, Fayetteville. Neonates of nonresistant and Bt-resistant (3 fold resistance to Dipel® ES (17,600 IU/mg)) soybean looper were obtained from the Insect Rearing Facility in the Entomology Department where they are maintained on artificial diet.

Influence of Cotton Leaf Location on Larval Survival and Growth

A thin layer of 4% agar water was added to the bottom of 30ml plastic cups (Solo Cup Company, Urbana, IL) to minimize desiccation of plant tissues. Nonresistant and Bt-resistant soybean looper neonates (200 larvae/treatment) were confined individually in these cups containing freshly excised upper, middle or lower leaves from nonBt- or Bt-cotton and reared at 28^0C for 48 h to determine their initial survivability. In each treatment, 25 larvae from each treatment were transferred to new cups containing respective tissues and reared until death or adult emergence. Leaves in the cups were changed on alternate days. Larval survival was recorded daily and larval weight was recorded after 10 days. The length of larval developmental period, percent larval survival to pupation, length of pupal developmental period and pupal weight were also recorded. The experiment was replicated four times.

Data Analyses

Larval mortality data were subjected to Arc Sine transformation. Data were analyzed following Analysis of Variance (ANOVA) and means separated by LSD (SAS system). Bioassay results were used to determine the LC_{50}s for doses using the Probit Procedures (SAS system). Linear relationships of larval weight with larval period, larval survivability, pupal weight, pupal developmental period and adult emergence were calculated.

Results

Survival of Bt-Resistant and Nonresistant Soybean Loopers

Survival of larvae from the nonresistant culture fed leaves from the lower, middle and upper nodes of nonBt- and Bt-cotton did not differ significantly through 10 days. Survival through pupation and adult emergence on either cotton was significantly higher in nonresistant larvae fed the middle leaves than those fed upper and lower leaves.

The survival for 10 days of Bt-resistant neonates did not differ on leaves of nonBt-cotton. On Bt-cotton survival was greater on middle leaves, except at 10 days it did not differ between larvae on upper and middle leaves. Survivability on both cotton varieties tended to be lowest in Bt-resistant larvae fed the lowest leaves. Larval survival through pupation and adult emergence in the Bt-resistant culture differed with cotton variety. Survival on the Bt-cotton was greater on middle leaves than other locations. Survival on the nonBt-cotton did not differ with upper and middle leaves, and both were greater than survival on lower leaves (Table 1). Across the cotton varieties, nonresistant larvae had significantly better survival than Bt-resistant larvae.

Growth and Development of Bt-Resistant and Nonresistant Soybean Loopers

In nonresistant soybean loopers, weight after 10 days on both cotton varieties was greatest when larvae were on middle leaves of nonBt-cotton. At each location, weight was greater on nonBt-cotton than Bt-cotton. Larval weight was positively correlated with larval survival at pupation ($r = 0.92$, $P<0.01$, $df = 4$) and adult emergence ($r = 0.98$, $P<0.01$, $df = 4$). Nonresistant larvae fed middle leaves of either nonBt or Bt-cotton had a significantly shorter larval developmental period than those fed upper or lower leaves. Larval weight was negatively correlated with the larval development period across either cotton varieties ($r = -0.99$, $P<0.01$, $df = 4$). The mean pupal weight and length of pupal developmental period of larvae fed nonBt- and Bt-cotton did not differ significantly. The pupal developmental period across all treatments was negatively correlated with larval weight ($r = -0.91$, $P<0.01$, $df = 4$). The larval and pupal developmental periods were positively correlated ($r = 0.95$, $P<0.03$, $df = 4$).

In the Bt-resistant soybean loopers, weight of larvae on both cotton varieties after 10 days differed with that of larvae fed middle leaves > upper leaves > lower leaves; except that of the larvae fed the upper and lower leaves of Bt-cotton did not differ significantly. However, for the developmental period, the trend was for it to be shortest on the middle leaves, and not differ between the other locations. The larval weight was positively correlated with larval survival through pupation ($r = 0.83$, $P<0.05$, $df = 4$) and adult emergence ($r = 0.81$, $P<0.05$, $df = 4$). The larval development period was negatively correlated with larval weight ($r = -0.93$, $P<0.01$, $df = 4$). The mean weight and developmental period of larvae fed nonBt- and Bt-cotton did not differ significantly. The pupal weight and developmental period of Bt-resistant larvae fed nonBt- or Bt-cotton did not differ significantly. The larval developmental period was negatively correlated with pupal weight ($r = -0.83$, $P<0.05$, $df = 4$). The length of pupal development period was negatively

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correlated with larval weight (r = -0.88, P<0.05, df = 4), but positively correlated with the length of larval developmental period (r = 0.84, P<0.05, df = 4) (Table 2). Across cotton varieties, in terms of growth, nonresistant larvae did not differ significantly from the Bt-resistant larvae.

Discussion

Our results showed that the survival of nonresistant soybean looper larvae for up to10 days did not differ significantly in Bt- and nonBt-cotton. Previous studies showed that survival of several plusiinae larval species including soybean looper was significantly higher on nonBt cotton than on Bt-cotton (Mascarenhas and Luttrell, 1997; Stapel et al. 1997; Adamczyk et al. 1998; Jenkins, et al. 1997; Parker, et al., 2000). However, survival at pupation or adult emergence was significantly higher in the nonresistant culture only. Mascarenhas et al. (1998) reported that soybean looper larvae collected from Bt-cotton are less susceptible *to B. thuringiensis* toxin.

Survivability of nonresistant and Bt-resistant soybean loopers differed with leaf location in both cotton varieties. Jenkins et al. (1993) reported that survival of *H. virescens* neonates on nonBt-cotton after 6 days of rearing ranged from 49% on old leaves to 88.5% in terminal leaves.

Larval weight was reduced in the nonresistant soybean looper culture on Bt-cotton. Growth of Bt-resistant larvae, however, was not affected by the cotton variety. M. Ashfaq and S. Young (unpublished data) also found an increase in the larval developmental time of nonresistant soybean looper on Bt-cotton. An extension in the larval developmental period on Bt-cotton was reported for beet armyworm by Stapel et al. (1997) and for fall armyworm by Adamczyk et al. (1998). Jenkins et al. (1993) reported that tobacco budworm larvae fed young leaves of nonBt-cotton had significantly higher weight than those fed old leaves. However, this weight gain was not significantly different from larvae on Bt-cotton. In our study, nonresistant soybean looper larvae fed leaves from the middle canopy had a significantly greater weight gain and shorter larval developmental period than those fed upper or lower leaves of either cotton. However, in the Bt-resistant culture, the affect of cotton leaf location on development was more notable in larvae fed lower leaves of Bt-cotton. Previous studies reported a reduction in pupal weight or length of developmental period in lepidopterous larvae fed Bt-cotton, but we did not observe a difference (Jenkins et al., 1993, Halcomb et al., 1996, Adamczyk et al., 1998). The pupal developmental period for both soybean looper strains did not differ significantly between the nonBt- and Bt-cotton. However, the significant negative correlation with larval weight and pupal development, and the positive correlation with larval and pupal developmental period suggest the influence of leaf location on pupal weight and developmental period.

In conclusion, these results indicated that cotton variety and leaf location significantly affect the survival and growth of nonresistant soybean looper. Survival and growth of Bt-resistant soybean looper was less affected by Bt-cotton than were nonresistant soybean loopers. The leaf from middle nodes on the cotton plants of both nonBt- and Bt-cotton may be most suitable for survival and growth for soybean loopers.

References

Adamczyk, Jr. J. J., Holloway, G. E., Church, G. E., Leonard, B. R., and J. B. Graves. 1998. Larval survival and development of the fall armyworm (Lepidoptera: Noctuidae) on normal and transgenic cotton expressing the *Bacillus thuringiensis* CryIA(c) δ-endotoxin. J. Econ. Entomol. 91: 539-545.

Felland, C. M., Porter, R. P., and H. N. Pitre. 1992. Soybean looper (Lepidoptera: Noctuidae) oviposition preference relative to plant development in soybean and cotton. J. Entomol. Sci. 27: 217-223.

Halcomb, J.L., Benedict, J. H., Cook, B., and D. R. Ring. 1996. Survival and growth of bollworm and tobacco budworm on nontransgenic and transgenic cotton expressing a CryIA insecticidal protein (Lepidoptera: Noctuidae). Environ. Entomol. 25: 250-255.

Jenkins, J. N., Parrott, W. L., McCarty, Jr. J. C., Callahan , F. E., Berberich, S. A. and W. R. Deaton. 1993. Growth and survival of *Heliothis virescens* (Lepidoptera: Noctuidae) on transgenic cotton containing a truncated form of the delta-endotoxin gene from *Bacillus thuringiensis*. J. Econ. Entomol. 86: 181-185.

Jenkins, J. N., McCarty, Jr. J. C., Buehler, R. E., Kiser, J., Williams, C. and T. Wofford. 1997. Resistance of cotton with δ-endotoxin genes from *Bacillus thuringiensis* var. *kurstaki* on selected lepidopteran insects. Agron. J. 89: 768-780.

Mascarenhas, R. N., Boethel, D J., Leonard, B. R., Boyd, M. L., and C. G. Clemens. 1998. Resistance monitoring to *Bacillus thuringiensis* insecticides for soybean loopers (Lepidoptera: Noctuidae) collected from soybean and transgenic bt-cotton. J. Econ. Entomol. 91: 1044-1050.

Parker, Jr. C. D., Mascarenhas, V.J., Luttrell, R. G., and K. Knighten. 2000. Survival rates of tobacco budworm (Lepidoptera: Noctuidae) larvae exposed to transgenic cottons expressing insecticidal protein of *Bacillus thuringiensis* Berliner. J. Entomol. Sci. 35: 105-117.

SAS Institute Inc. 1988. SAS/STAT User's Guide, Release 6.03 Edition. Cary, NC. 1028 pp.

Sprenkel, D., and F. Johnson. 1998. Insect monitoring and control in cotton. Coop. Ext. Serv. University of Florida.. P 37.

Stapel, J. O., Waters, D. J., Ruberson, J. R., and W. J. Lewis. 1998. Development and behavior of *Spodoptera exigua* (Lepidopter: Noctuidae) larvae in choice test with food substrates containing toxins of *Bacillus thuringiensis*. Biological Control 11: 29-37.

Table 1. Survival of Nonresistant and Bt-resistant Soybean Loopers on nonBt and Bt- Cotton.

Leaf locations	Survivability (%) at 48 h	Survivability (%) at 10 days	Pupation	Adult emergence
Nonresistant soybean looper				
NonBt-cotton				
Upper	64.5[ns]	72.2 [ns]	57.0 bc [1]	56.9 bc [1]
Middle	68.4	80.8	68.4 a	68.4 a
Lower	68.1	82.2	58.4 bc	53.6 cd
Bt-cotton				
Upper	69.8	78.9	47.9 d [1]	47.2 d [1]
Middle	62.8	82.2	63.8 ab	61.8 b
Lower	64.1	74.0	53.1 cd	48.6 d
Bt-resistan soybean looper				
NonBt-cotton				
Upper	66.4 b [1]	78.9 abc [1]	60.9 a [1]	59.2 a [1]
Middle	66.4 b	74.6 abc	59.2 a	56.8 a
Lower	57.1 b	69.4 bc	42.1 c	39.9 c
Bt-cotton				
Upper	54.6 bc	82.1 ab	46.4 b	45.7 b
Middle	90.0 a	86.8 a	58.4 a	57.6 a
Lower	42.7 c	67.9 c	43.6 bc	42.1 bc

[ns] Not significantly different (P>0.05).

[1] Means in a column (within an insect culture) followed by same letter(s) are not significantly different (P<0.05).

Table 2. Growth of nonresistant and Bt-resistant Soybean Loopers on NonBt and Bt-Cotton.

Leaf location	Larval wt. (mg)	Larval period (days)	Pupal wt. (mg)	Pupal period (days)
Nonresistant Soybean Looper				
NonBt-cotton				
Upper	69.1 b [1]	18.3 bc[1]	193.6 [ns]	6.2 ab[1]
Middle	92.9 a	16.3 d	201.5	6.0 b
Lower	60.1 cb	18.5 abc	180.0	6.2 ab
Bt-Cotton				
Upper	45.8 cd	19.5 ab	180.1	6.2 ab
Middle	70.6 b	17.7 dc	184.3	6.1 ab
Lower	42.2 d	20.1 a	187.0	6.3 a
Bt-resistant soybean looper				
NonBt-cotton				
Upper	42.4 b [1]	18.2 bc [1]	200.3 [ns]	6.1 [ns]
Middle	63.7 a	17.5 c	196.2	6.0
Lower	27.0 cd	20.3 ab	177.0	6.2
Bt-Cotton				
Upper	31.1 bc	20.9 a	169.6	6.1
Middle	44.1 b	18.3 bc	186.3	6.0
Lower	17.4 d	21.6 a	180.1	6.3

[ns] Not significantly different (P>0.05).

[1.] Means in a column (within an insect culture) followed by the same letter(s) are not significantly different (P<0.05).