VIRUS PRODUCTION AND GROWTH OF BOLLWORM AND TOBACCO BUDWORM ON FRUITING STRUCTURES OF COTTON

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Abstract

Effects of cotton fruiting on bollworm, *Helicoverpa zea* (Boddie) and tobacco budworm, *H. virescens* (F.) larval growth, survivorship, length of pupal developmental period, pupal weight, susceptibility to *H. zea* nucleopolyhedrovirus (HzNPV) and production of virus in HzNPV-infected larvae were studied. Larval weight of both species after 10 days of rearing, varied significantly ($P < 0.05$) and was in the order of artificial diet > flower-anthers > leaves > squares > sq. bracts. The length of larval developmental period, larval survivability, pupal weight and length of pupal developmental period was affected similarly. All of these were positively correlated with larval weight, except that the length of larval developmental period was negatively correlated. Mortality of HzNPV-treated bollworm or tobacco budworm larvae fed on square, sq. bracts and flower anthers ranged from 64.8 to 67.9% and 50.5 to 60.1%, respectively, and did not differ significantly among the tissues, indicating that dietary variation in cotton fruiting structures did not affect larval susceptibility to HzNPV. The number of viral occlusion bodies (OBs) produced by a larva fed square, sq. bracts or flower-anthers, respectively, were 2.0×10^7, $2.2 \times 10^7$and 6.6×10^7 for bollworm and 2.0×10^7, 1.0×10^7, and $9.3 \times 10^7$for tobacco budworm. In both species, larvae fed on flower-anthers produced significantly ($P<0.05$) higher number of OBs than those on squares or sq. bracts. In both species, the number of OBs produced by an infected larva fed different fruiting structures was positively correlated with the weight gained by a healthy larva fed on the same type of tissues. These results indicate that dietary variation in different reproductive structures of cotton directly affects bollworm and tobacco budworm larval growth and development, and indirectly affects the production of virus by HzNPV-infected larvae.

Introduction

The bollworm, *Helicoverpa zea* (Boddie) and tobacco budworm, *H. virescens* (F.) are major pests of cotton, *Gossypium hirsutum* L. in the United States (Stadelbacher, 1979). *H. zea* nucleopolyhedrovirus (HzNPV) has potential for use as a viral pesticide against bollworm and tobacco budworm on cotton and other agricultural crops (Yearian and Young, 1982). Larval mortality resulting from baculovirus infection varies significantly with host plants (Fuxa, 1982; Richter et. al., 1987; Keating and Yendol, 1987; Alvarez and Garcia, 1992; Forschler et. al., 1992; Duffey et al., 1995; Ali et. al., 1998a) as well as within a plant (Ali et. al. (1998a). Ali et. al. (1998a) also showed that susceptibility of bollworm and tobacco budworm larvae to HzNPV infection differeed on vegetative and reproductive tissues of host plants. They also (Ali, et. al., 1998b) reported that bollworm and tobacco budworm larvae fed cotton leaves produced higher numbers of HzNPV than larvae on reproductive tissues, such as squares. In this investigation, the influence of the cotton plant's phenological changes in fruiting structures on several biological parameters of bollworm and tobacco budworm, as well as its effects on larval susceptibility to HzNPV and production of virus by an infected larva are reported.

Materials and Methods

Cotton, *Gossypium hirsutum* L. (cv. Stoneville 474) was grown on research plots at the Agricultural Experiment Station, University of Arkansas, Fayetteville following standard agronomic practices. Bollworm and tobacco budworm neonates were obtained from the Insect Rearing Facility in the Entomology Department where they are maintained on artificial diet (Burton, 1969).

Cotton Phenology Effect on Larval Growth

Bollworm or tobacco budworm neonates were placed individually in 30ml plastic cups (Solo Cup Company, Urbana, IL) containing field grown cotton tissues of either 1) terminal leaf, 2) young square with bract, 3) square bract, 4) newly bloomed flower or 5) artificial diet and reared at 28^0C until pupation (25 larvae/treatment). A thin layer of 4% agar water was added to the bottom of the cups to minimize desiccation of tissues. Tissues in the cups were changed on alternate days. Neonates fed squares tended to feed on bracts first and as they grew older bored into the square. The neonates fed on flowers tended to feed flower-petals initially, and later moved to flower anthers. Larval survival was recorded daily and larval weight was recorded after 10 days of rearing. Data on the length of larval developmental period, larvae survive to pupation, length of pupal developmental period and pupal weight were recorded.

Cotton Phenology Effect on Larval Susceptibility to HzNPV

Young squares, square bracts, and newly bloomed flowers were collected separately and placed individually in 500-ml plastic containers (Fabri-Kal Co., Kalamazoo, MI) containing moistened filter paper (Whatman No 1) in the bottom. Twenty neonates of bollworm or tobacco budworm were placed in each container and reared at 28^0C to the second instar for the bioassays. Twenty five larvae of each species were treated as second instar by feeding for 24h on 4 mm

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 2:1353-1356 (2000)
National Cotton Council, Memphis TN

diameter disks of the tissues on which 0.1 µl of an aquous suspension containing 300 OBs of HzNPV (Elcar, Sandoz Crop Protection, Des Plaines, IL) in 1% Triton X-100 had been applied. The treated disks for larvae on square and sq. bracts were cut from bracts and those for larvae fed blooms from flower petals. The bioassay arena was made by embedding plastic grids in a Petri-dish with a layer of 4% agar-water to create 25 individual cells. A HzNPV-treated tissue-disk, and a single second instar larva was placed in each cell (25 larvae/replicate). Each of the three treatments was replicated four times and each replicate consisted of one bioassay arena. A second instar bollworm or tobacco budworm larva reared either on square, square bract, or flower tissues was confined for 24 h in a cell (25 larvae/replicate) containing the tissues on which it was being reared as control. After 24 h, 20 of the larvae that consumed the entire disk for each replicate were transferred individually to 30-mm clear plastic cups containing 4% agar-water and the respective tissues and reared for 10 days. Tissue in the cup was replaced with fresh tissue on alternate days. Larval survival was recorded daily. Cadavers of HzNPV-infected larvae were collected and preserved at 20^0 C in 1.5ml plastic Eppendorf microcentrifuge tubes. Ten cadavers were randomly selected in each treatment for counting OBs. Individual cadavers were prepared for counting by placing in a 1.5ml microcentrifuge tube, macerating and homogenizing with a plastic pestle. Distilled water was added to make a 1.0ml aliquot. Counts of viral OBs were made by diluting the suspension in 0.1% Triton X-100 solution and counting with an improved Neubauer hemacytometer under a phase contrast microscope.

Data Analyses

HzNPV-related larval mortality data were subjected to Arc Sine transformation. The significance of phenological variations of cotton fruiting structures on larval weight after 10 days, percent larval survival and, length of larval period, pupal weight, length of pupal period, HzNPV-related larval mortality and OBs produced by an infected larva was analyzed following Analysis of Variance Procedures (SAS system). Larval survivorship, length of larval developmental period, pupal weight and length of pupal developmental period, and number of OBs produced by an infected larva were plotted against the larval weight to calculate their logarithmic regression relationships with the larval weight.

Results

Cotton Phenology Effect on the Growth

Bollworm larval weights after 10 days of rearing ranged from 76.7 to 610.1 mg and were in the order of artificial diet > flower > leaves > squares > sq. bract (Table 1). Larvae fed on artificial diet had significantly higher weight than those fed on plant tissues. Among the plant tissues, larvae fed on flower-anthers had significantly higher weight than those fed on other tissues. Larvae fed on leaves or squares had a similar weight that was greater than that of those fed on sq. bracts. The length of larval developmental periods varied significantly among the diets and was negatively ($R^2 = 0.68$, $y = -7.5\text{Ln}(x) + 58.70$) correlated with larval weights. Larvae fed on sq. bracts had the longest (32.7 days) larval developmental period and those fed on artificial diet had shortest (12.0 days) larval developmental period. Larval survival to pupation varied and showed a positive correlation ($R^2 = 0.36$, $y = 10.70\text{Ln}(x) + 26.40$) with the larval weight. Larvae fed on artificial diet and leaf tissues had the highest survival rates (96.3%) among the diets. Survivability of larvae fed the other three tissues ranged from 61.3 to 77.5% and was statistically similar. Pupal weights varied among the diets and were significantly ($P<0.05$) positively correlated ($R^2 = 0.94$, $y = 101.74\text{Ln}(x) + 224.53$, df = 3) with larval weights. Pupal developmental periods among the diets varied and showed a significant ($P<0.05$) positive correlation ($R^2 = 0.83$, $y = 0.24\text{Ln}(x) + 8.92$, df = 3) with the larval weight. Larvae fed artificial diet had a longer pupal developmental period (10.5 days) than those fed on plant tissues. The length of pupal developmental period of larvae fed on leaves, squares, sq. bracts or flower-anthers ranged from 9.9 to 10.1 days and was statistically similar.

Development of the tobacco budworm was similar to that of bollworm (Table 2). Larval weight at 10 days rearing ranged from 413.0 mg on artificial diet to 29.5 mg sq. bracts and varied significantly among the diets. The larval developmental period ranged from 11.5 to 28.7 days among the diets and was significantly ($P<0.05$) negatively correlated ($R^2 = 0.93$, $y = -6.23\text{Ln}(x) + 47.72$, df = 3) with the larval weights. Larval survival to pupation ranged from 62.5 to 96.3% among the diets and was positively correlated ($R^2 = 0.70$, $y = 13.55\text{Ln}(x) + 16.68$, df = 3) with the larval weights. Pupal weight ranged from 145.1 to 328.4 mg among the diets and was significantly ($P<0.05$) positively correlated ($R^2 = 0.89$, $y = 73.85\text{Ln}(x) - 135.99$, df = 3) with the larval weights. Larvae fed on the artificial diet had largest pupal weight (328.4 mg) followed by flower-anthers > leaves > squares and > sq. bracts. Length of pupal period ranged from 10 to 10.5 days among the diets and were significantly ($P<0.05$) positively correlated ($R^2 = 0.96$, $y = 0.19\text{Ln}(x) + 9.42$, df = 3) with the larval weights. Larvae fed on artificial diet had the longest pupal developmental period followed by flower-anthers > leaves > squares and > sq. bracts.

Cotton Phenology Effect on Mortality and Virus Production

Mortalities of HzNPV-treated bollworm and tobacco budworm larvae fed on squares, sq. bracts and flower-anthers ranged from 64.8 to 67.9% and 50.5 to 60.1%, respectively and were not statistically different in either species. The number of viral OBs produced by a bollworm larva fed on square, sq. bracts and flower-anthers was 2.0×10^7, 2.2×10^7

and 6.6 X 10^7, respectively, while these figures for tobacco budworm were 2.0 X 10^7, 1.0 X 10^7 and 9.3 X 10^7, respectively. In both species, the number of OBs produced by a larva fed on flower- anthers was significantly higher than the number of OBs produced by a larva fed on squares and sq. bracts and was positively correlated with the larval weights (Table 3).

Discussion

Growth and development in both species was better on artificial diet than on tissues from the cotton plant. Larvae fed on flower-anthers grew better, had shorter larval developmental time, higher survival rate, longer pupal developmental time and greater pupal weight than those fed on other plant tissues. Among the other plant tissues, sq. bracts were the least satisfactory diet. These results suggest differences in dietary nutritional factors among different cotton tissues that are responsible for the above mentioned variation. The nutritional profile of the cotton variety, ST 474 used in the present study is not known. However, studies have shown that cotton contains many naturally occurring phenolic anti-nutritive compounds that inhibit bollworm and tobacco budworm larval growth (Lukefahr and Martin, 1966; Shaver, et. al., 1977; Chan, et. al., 1978; Jenkins, et. al., 1983; Zummo, et. al., 1983; Zummo, et. al., 1984; Hedin, et. al., 1991, 1992). Differences in essential nutrients, such as amino acid may also lead to differences in larval growth (Hedin, et. al., 1991). The relative quantity and quality of these nutritive factors as well as the anti-nutrient compounds in plant tissues may vary (Zummo, et al., 1984; Hedin, et. al., 1991). Thus, in the present study the gradual reduction in weights of larvae that fed on flower-anthers, leaves, square and sq. bracts could be explained by the gradation of phenolics in those plant tissues Zummo, et al., 1984; Hedin, et. al., 1991) as well as the availability of essential nutrients, particularly the amino acids in different plant tissues (Hedin, et. al., 1991).

Mortality of HzNPV-treated bollworm or tobacco budworm grown on a diet of square, sq. bract or flower-anther tissues did not differ. However, the number of OBs produced by an HzNPV-infected larvae did differ with diet as it was greater in larvae fed on flower-anthers than in those fed either square or sq. bract tissues. Previous studies showed that the number of OBs produced by NPV-infected *H. armigera* or *Mamestra brassicae* (L.) larvae is directly correlated with the larval age and weight at death (Evans, 1981; Teakle, et al., 1985; Teakle and Byrne, 1989). Even though the larvae used in our study were treated with HzNPV as second instar, we observed that larvae fed flower-anthers grew faster and were generally larger at death than on squares or sq. bracts. Thus, larvae fed on flower-anthers produced more OBs than those on squares and sq. bracts. We previously reported that the number of OBs produced by an HzNPV-infected bollworm or tobacco budworm larvae fed on vegetative (leaf) tissues of cotton and soybean were significantly ($P<0.05$) higher than on reproductive (squares) tissue (Ali, et al., 1998b).

Summary

These results indicate that cotton phenology significantly affects the development of bollworm and tobacco budworm with differences in larval weight, larval survivability, length of larval developmental period, pupal weight and length of pupal developmental period. Bollworm and tobacco budworm larvae fed leaf, square, sq. bract or flower-anther tissues showed degration in larval weight from flower-anthers > leaves > squares > sq. bracts. Length of larval developmental period was negatively correlated with larval weight whereas larval survivability, pupal weight and length of pupal developmental period were positively correlated with larval weight. Second instar bollworm and tobacco budworm larval susceptibility to HzNPV on different reproductive tissues was similar. However, HzNPV-infected larvae of either species fed flower-anthers produced significantly more OBs than those fed squares and sq. bracts. These results indicate that nutritional variation in different reproductive structures of cotton directly affects growth and development of bollworm and tobacco budworm, and indirectly affects the production of HzNPV by infected larvae.

Acknowledgment

This manuscript is based upon work supported in part by USDA Grant (95-34103-1542) and published with the approval of the Director, Arkansas Agricultural Experiment Station.

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Table 1. Cotton Phenology Effects on the Growth of bollworm[1]

	Leaves	Squares	Sq. bracts	Flowers	Art. diet
Larval[2] weight (mg)	110.5 ± 4.5c[3]	104.2 ± 5.1c[3]	76.7 ± 3.3d[3]	246.2 ± 9.4b[3]	610.1 ± 9.2a[3]
Larval period (days)	19.4 ± 0.2c	20.0 ± 0.2b	32.7 ± 0.4a	16.7 ± 0.2d	12.0 ± 0.1c
Larval survial (%)	96.3 ± 2.4a	75.0 ± 6.4b	61.3 ± 1.3b	77.5 ± 1.4b	96.3 ± 2.4a
Pupal weight (mg)	261.3 ± 5.9bc	252.5 ± 6.3cd	224.1 ± 8.9d	297.6 ± 5.9b	447.5 ± 6.7a
Pupal period (days)	10.1 ± 0.1b	10.1 ± 0.1b	9.9 ± 0.1b	10.1 ± 0.1b	10.5 ± 0.2a

[1]Reared at 28 ^{0} C
[2]After 10 days of rearing
[3]Figures in column followed by same letter(s) are significantly different (P<0.05)

Table 2. Cotton Phenology Effects on the Growth of Tobacco Budworm[1]

	Leaves	Squares	Sq. bracts	Flowers	Art. diet
Larval[2] weight (mg)	94.5 ± 5.2b[3]	64.0 ± 3.9c[3]	29.5 ± 3.0d[3]	99.8 ± 2.8b[3]	413.0 ± 8.2a[3]
Larval period (days)	18.7 ± 0.2c	20.4 ± 0.3b	28.7 ± 0.3a	17.8 ± 0.2c	11.5 ± 0.1d
Larval survial (%)	92.5 ± 2.5a	65.0 ± 5.4c	62.5 ± 1.4c	75.0 ± 2.9b	96.3 ± 2.4a
Pupal weight (mg)	1873 ± 3.2b	145.1 ± 3.7c	146.8 ± 2.5c	190.2 ± 2.5b	328.4 ± 2.5a
Pupal period (days)	10.3 ± 0.1ab	10.2 ± 0.1bc	10.0 ± 0.1c	10.3 ± 0.1bc	10.5 ± 0.1a

[1]Reared at 28 ^{0} C
[2]After 10 days of rearing
[3]Figures in column followed by same letter(s) are significantly different (P<0.05)

Table 3. Cotton Phenology Effects on the Mortality and Number of Viral Occlusion Bodies (OB) Produced by HzNPV -Treated Heliothine Larvae [1]

	Bollworm		Tobacco budworm	
	Mortality (%)	OB produced (No X 10^7)	Mortality (%)	OB produced (No X 10^7)
Square	64.8 ± 3.9a[2]	2.0 ± 0.4b[2]	60.1 ± 11.8a[2]	2.0 ± 0.6b[2]
Sq bracts	65.1 ± 2.7a	2.2 ± 1.1b	50.5 ± 10.1a	1.0 ± 0.2b
Flower	67.9 ± 2.7a	6.6 ± 2.4a	60.1 ± 1.4a	9.3 ± 3.3a

[1]Larvae were treated as second instars with a dose of 3 X 10^2 OBs/larva
[2]Figures in column followed by same letter(s) are significantly different (P<0.05)