EFFECTS OF HOST PLANTS ON THE VIRAL PRODUCTION IN BUDWORM AND BOLLWORM

M. I. Ali, G. W. Felton and S. Y. Young
Department of Entomology, University of Arkansas
Fayetteville, AR

Abstract

Effects of host plant inter-and intra-specific variation of cotton, soybean and clover on the yield and infectivity of occlusion bodies (OBs) of *Helicoverpa zea* nucleopolyhedrovirus (HzNPV) in tobacco budworm, *Heliothis virescens* (F.) and bollworm, *H. zea* (Boddie) larvae were studied. The number of OBs produced by *H. zea* or *H. virescens* larvae fed on vegetative tissues was significantly ($P<0.05$) higher than by larvae fed on reproductive tissues in all tested plant species. The total number of OBs produced by *H. zea* or *H. virescens* larvae on vegetative or reproductive tissues of cotton, soybean and clover varied significantly. LC_{50} values for both species fed HzNPV were higher when the virus was produced in larvae fed on reproductive tissues than on vegetative tissues on all host plants for *H. virescens* but only for soybean in *H. zea*. Thus the infectivity of the OBs produced by *H. zea* and *H. virescens* on reproductive tissues was reduced compared to OBs produced in larvae on vegetative tissues. This reduction in infectivity of OBs in larvae fed on reproductive tissues was most prominent in *H. virescens* on cotton, followed by soybean and clover. In conclusion, viral production and viral infectivity in heliothine larvae was affected by both tissue and plant species.

Introduction

The bollworm, *Helicoverpa zea* (Boddie) and tobacco budworm, *Heliothis virescens* (F.) are major pests of cotton in the United States (Stadelbacher, 1979). *H. zea* nucleopolyhedrovirus (HzNPV) has potential for use as a viral pesticide against the *H. zea* and *H. virescens* on cotton and other agricultural crops (Yearian and Young, 1982). Larval mortality resulting from baculovirus infection varies significantly on different 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*., 1997). Ali *et al*. (1997) showed that susceptibility of *H. zea* and *H. virescens* larvae to HzNPV infection on vegetative and reproductive tissues of host plants also varied significantly. The mechanistic relationship of these variations in susceptibility of herbivores to entomopathogens is not known. It is reported that the infectivity of baculovirus to lepidopterous larvae is dependent on the phenolic composition (Keating and Yendol, 1987) and the foliar oxidative enzymes, such as polyphenol oxidase and peroxidase (Hoover *et al*., 1997) of the host plant. Effect of host plant on the production and infectivity of viral inocula by the host insect is not known.

In this investigation, the influence of host plant inter- and intra-specific variation of two cultivated (cotton and soybean) and a non-cultivated host (crimson clover) of *H. zea* and *H. virescens* on production of occlusion bodies (OBs) of HzNPV and their infectivity in *H. zea* and *H. virescens* larvae is reported.

Materials and Methods

Laboratory germinated seeds of cotton, *Gossypium hirsutum* L. (cv. Stoneville 213); soybean, *Glycine max* L. Merrill (cv. Forrest); and crimson clover, *Trifolium incarnatum* L. (hereafter clover) were grown in 2-L plastic pots filled with Redi-earth Peat-Lite Mix soil mixture (Scotts-Sierra Horticultural Products Company, Marysville, OH) in a greenhouse. Pots were arranged in a randomized complete block design. Plants were watered every day . Fertilizer (N:P:K = 20:20:20) was applied weekly. Greenhouse conditions were : (1) 14-h photophase, using high-pressure sodium light and (2) day and night temperatures of 33 ± 2^0C and 20 ± 2^0C, respectively). *H. zea* or *H. virescens* neonates were obtained from the University of Arkansas Insect Rearing Facility and reared either on vegetative (leaves) or reproductive (squares for cotton and petals for soybean and clover) tissues in 30-cm clear plastic cups to the second instar.

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. Disks were cut from the vegetative and reproductive tissues of the respective host plants, and 0.1µl HzNPV (Elcar®, Sandoz Crop Protection, Des Plaines, IL, USA) in 1% Triton X-100 was applied to each disk. Dosages were 10, 30 and 100 OBs/larva for clover, soybean and cotton, respectively. Each treatment was replicated four times and for each replicate one bioassay arena was prepared. A second instar was confined to a cell and allowed to feed on a leaf disk for 24 h. After 24 h, 20 of the larvae that consumed the entire leaf disks for each replicate were individually transferred into 30-mm clear plastic cups containing agar-water and either vegetative or reproductive tissues of the respective hosts and reared for 10 days. Thereafter the tissues in cups were replaced with fresh tissues on alternate days. Larval survival was recorded daily. Instars of moribund larvae were determined by examining their head capsules (all were second instar) and preserved at -20° C in 1.5 ml plastic Eppendorf microcentrifuge tubes. Ten cadavers from HzNPV-infected larvae were randomly selected in each treatment for counting of OBs. Individual cadavers were placed in a 1.5 ml microcentrifuge tube, macerated and homogenized by a plastic pestle, and distilled water was added to make 1.0 ml aliquot. For counting of OBs, 1.0 µl aliquot was diluted 10 to 20 times in 0.1% Triton X-100 solution. For each sample, four counts were made using an improved

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 2:1337-1339 (1998)
National Cotton Council, Memphis TN

Neubauer hemacytometer under a phase contrast microscope.

For each sample, a 0.5 ml aliquot was used to prepare serial doses at a series of concentrations (1×10^3, 3.3×10^3, 1×10^4 and 3.3×10^4 OBs/ml) in distilled water. A 100 µl Eppendorf pipette was used to apply 0.1 ml of each solution on artificial diet in 30-ml clear plastic cups. For the control, 0.01 ml of distilled water was applied. The solutions were spread uniformly over the diet surface by blowing air gently and allowing it to dry for 30 min. Individual neonates of *H. zea* or *H. virescens* were confined in cups and reared at $29.5^0 \pm 1.0$ C for 14 d. For each concentration 25 larvae were tested. Larval survival was recorded on alternate days.

The significance of host plant intra-specific (vegetative and reproductive stages) and inter- specific (cotton, soybean and clover) variation in OB production by *H. zea* and *H. virescens* was analyzed following Analysis of Variance Procedures (SAS system). Bioassay results of OB infectivity were used to determine LC_{50}s using the Probit Procedures (SAS system).

Results and Discussion

OBs Produced by HzNPV-Infected *H. Zea* Larvae

The number of OBs produced by larvae fed on vegetative tissues of cotton, soybean and clover were 57.7×10^6, 84.0×10^6 and 81.0×10^6 OBs/larva, respectively, in comparison with 26.5×10^6, 58.2×10^6 and 75.3×10^6 OBs/larva, respectively on reproductive tissues. The number of OBs produced by larvae on vegetative tissues of cotton and soybean were significantly ($P<0.05$) higher than on reproductive tissue, but OB production in larvae fed on clover did not differ significantly ($P<0.05$). The number of OBs produced by larvae on vegetative tissues of cotton, soybean and clover were statistically similar. The number of OBs produced by larvae on reproductive tissues of cotton was significantly ($P<0.05$) lower than when larvae were fed on soybean or clover, but the latter did not differ significantly (Table 1).

OBs Produced by HzNPV-Treated *H. Virescens* Larvae

The number of OBs produced by larvae fed on vegetative tissues of cotton, soybean and clover were 26.1×10^6, 173.7×10^6, and 50.4×10^6 OBs/larva, respectively, in comparison with 9.1×10^6, 28.1×10^6 and 38.4×10^6 OBs/larva on reproductive tissues. The number of OBs produced by larvae on vegetative tissues of cotton and soybean were significantly ($P<0.05$) higher than on reproductive tissue. Larvae fed on vegetative tissue of host plants differed significantly ($P<0.05$) with the number of OBs on soybean>clover>cotton. The number of OBs produced by larvae on reproductive tissues of cotton, soybean or soybean did not differ significantly (Table 1).

Bioassays of OBs Produced by *H. Zea* Larvae

The LC_{50}s for HzNPV produced in 2nd instar fed on vegetative tissues of cotton, soybean and clover were, respectively, 9.4×10^3, 6.8×10^3, and 1.3×10^3 OBs/larva in comparison with 3.4×10^3, 15.6×10^3 and 1.3×10^3 OBs/larva in larvae fed on reproductive tissues. However, the LC_{50}s of OBs did not differ significantly between larvae fed vegetative or reproductive tissues for any of the plant species studied. The LC_{50}s of OBs produced in larvae fed vegetative and reproductive tissues in clover were significantly ($P<0.05$) less than that in cotton and soybean (Table 2).

Bioassays of OBs Produced by *H. Virescens* Larvae

The LC_{50}s for HzNPV produced in 2nd instar *H. virescens* fed on vegetative tissues of cotton, soybean and clover were, respectively, 11.3×10^3, 11.0×10^3, and 5.6×10^3 OBs/larva in comparison with 17.6×10^6, 31.0×10^3 and 11.6×10^3 OBs/larva in larvae fed on reproductive tissues. The LC_{50}s for HzNPV from larvae fed reproductive tissues in comparison with vegetative tissues were over 2, 3 and 1500 fold higher and significantly ($P>0.05$) greater than in clover, soybean and cotton, respectively. The LC_{50}s for *H. virescens* fed reproductive tissues were significantly ($P<0.05$) higher than for vegetative tissues of all plants. The LC50s of HzNPV produced in larvae fed reproductive tissues were in the order of cotton>soybean>clover ($P<0.05$). When larvae were fed virus on vegetative tissues, the LC_{50}s on cotton and soybean did not differ, but LC_{50}s of virus from larvae on clover were significantly lower than that of either of the other hosts (Table 2).

Summary

These results suggest that type of tissue or host plant species on which larvae feed can result in differences in virus production. Also, in both insect species studied, the number of OBs produced was higher in the vegetative than reproductive tissues. Host plants and the type of tissue on which larvae feed may also affect infectivity of virus produced. Infectivity of HzNPV in *H. virescens* was consistantly lower when larvae were fed on the reproductive tissue of plants. Otherwise differences in HzNPV infectivity, when it occured was not consistant between plants or insects tested. In general, HzNPV production and infectivity tended to be lowest in larvae fed on cotton. Previously, we (Ali *et al*., 1998) showed that *H. zea* or *H. virescens* larvae fed on vegetative tissues of soybean, clover or cotton were more susceptible to HzNPV than those fed on reproductive tissues.

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. We thank Drs. T. J. Kring and D. C. Steinkraus, Department of Entomology, University of Arkansas,

Fayetteville for allowing use of their facilities for counting the OBs.

References

Ali, M. I., G. W. Felton, Meade, T. and S. Y. Young 1997. Influence of host inter-specific and intra-specific variation on the susceptibility of heliothines to a baculovirus. Biologic Contr. (submitted).

Alvarez, C. S. and R. O. Garcia 1992. The influence of host plant on the susceptibility of *Spodoptera littoralis* (Boisd.) (Lep., Noctuidae) larvae to *Spodoptera littoralis* NPV (Baculoviridae, *Baculovirus*). J. Appl. Entomol. 114: 124-130.

Duffey, S. S., Hoover, K., Bonning, B. and B. D. Hammock 1995. The impact of host plant on the efficacy of baculoviruses. Rev. Pestic. Toxicol. 3: 137-275.

Forschler, B. T., Young, S. Y. and G. W. Felton 1992. Diet and the susceptibility of *Helicoverpa zea* (Noctuidae: Lepidoptera) to a nuclear polyhedrosis virus. Environ. Entomol. 21: 1220-1223.

Fuxa, J. R. 1982. Prevalence of viral infections in populations of fall armyworm, *Spodoptera frugiperda* in Southern Louisiana. Environ. Entomol. 11: 239-242.

Hoover, K., Yee, J. L., Schultz, C. M., Rocke, D. M., Hammock, B. D. and S. S. Duffey 1997b. Effects of plant identity and chemistry on the efficacy of baculovirus against *Heliothis virescens*. J. Chem. Ecol. (submitted).

Keating, S. R. and W. G. Yendol 1987. Influence of selected host plants on gypsy moth (Lepidoptera: Lymantridae) larval mortality caused by a baculovirus. Environ. Entomol. 16: 459-462.

Richter, A. R., Fuxa, J. R. and M. A. Fattah 1987. Effect of host plant on the susceptibility of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to a nuclear polyhedrosis virus. Environ. Entomol. 16: 1004-1006.

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

Stadelbacher, E. A. 1979. *Geranium dissectum*: an unreported host of tobacco budworm and bollworm and its role in their seasonal and long term population dynamics in the Delta of Mississippi. Environ Entomol. 8:1153-1156.

Yearian, W. C., and S. Y. Young. 1983. Control of insect pests of agricultural importance by viral pesticides, 387423 pp. In Microbial and Viral Pesticides (E. Kurstak, Ed.), Marcel Dekker, Inc. New York.

Table 1. Number of occlusion bodies produced by heliothine larvae on different host plants.

Number of occlusion bodies produced (X 10^6)			
Plant tissue type	Plant species		
	Cotton	Soybean	Clover
	Helicoverpa zea		
Vegetative	57.7 a^1A^2	84.0 a^1A^2	81.0 a^1A^2
Reproductive	26.5 bB^2	58.2 bA^2	75.3 aA^2
	Heliothis virescens		
Vegetative	26.1 a^1C^2	173.7 a^1A^2	50.4 a^1B^2
Reproductive	9.1 bA^2	28.1 bA^2	38.4 aA^2

[1] Means in a column in each insect species, followed by same lower case letters are not significantly different (P>0.05, by LSD).
[2] Means in a row among the plant species, in each tissue, followed by same upper case letters are not significantly different (P>0.05, by Paired T-Test).

Table 2. LC_{50}s of OBs produced by respective second instar heliothine fed on vegetative or reproductive tissues of different host plants[1]

Plant/tissues	LC_{50} OB/ml[2]	95% Fiducial Limit		Slope	Intercept
		Lower	Upper		
	Helicoverpa zea				
Cotton					
Vegetative	9.4 X 10^3	4.8 X 10^3	23.6 X 10^3	1.3	-5.3
Reproductive	3.4 X 10^3	1.4 X 10^3	6.4 X 10^3	1.5	-5.3
Soybean					
Vegetative	6.8 X 10^3	5.8 X 10^3	8.1 X 10^3	1.2	-4.5
Reproductive	15.6 X 10^3	6.5 X 10^5	183.5 X 10^3	1.0	-4.0
Clover					
Vegetative	1.3 X 10^3	1.0 X 10^3	1.5 X 10^3	1.8	-5.6
Reproductive	1.3 X 10^3	1.1 X 10^3	1.6 X 10^3	1.7	-5.4
	Heliothis virescens				
Cotton					
Vegetative	11.3 X 10^3	4.7 X 10^3	69.6 X 10^3	0.9	-3.7
Reproductive	17.6 X 10^6	5.1 X 10^4	90.0 X 10^{18}	0.3	-2.2
Soybean					
Vegetative	11.0 X 10^3	8.8 X 10^3	14.2 X 10^3	0.9	-3.5
Reproductive	31.0 X 10^3	21.0 X 10^3	54.6 X 10^3	0.9	-4.2
Clover					
Vegetative	5.6 X 10^3	4.2 X 10^3	7.4 X 10^3	0.7	-2.5
Reproductive	11.6 X 10^3	9.1 X 10^3	15.6 X 10^3	0.8	-3.2

[1] LC_{50}s determined using heliothine neonates fed on artifical diet.
[2] LC_{50} is in Occlusion bodies (OB)/ml of HzNPV suspension assayed.