# INFLUENCE OF HOST PLANT ON OCCLUSION BODY PRODUCTION AND INFECTIVITY OF A BACULOVIRUS

**M. Ibrahim Ali, S. Y. Young and G.W. Felton**
**Department of Entomology**
**University of Arkansas**
**Fayetteville, AR**
**T. Meade**
**Mycogen**
**Indianapolis, IN**
**D. A. Streett**
**Southern Insect Management Laboratory**
**USDA-ARS**
**Stoneville, MS**
**R. W. McNew**
**Agricultural Statistics Lab**
**University of Arkansas**
**Fayetteville, AR**

## Abstract

Intra- and inter-specific effects of cotton, soybean and clover on the survival time, yield and infectivity of occlusion bodies (OBs) of the *Helicoverpa zea* nucleopolyhedrovirus (HzNPV) in infected heliothine, *Helicoverpa zea* (Boddie) and *Heliothis virescens* (F.), larvae were studied. Survival time of HzNPV-infected *H. zea* fed on vegetative tissues of clover was significantly higher than on reproductive tissues. Across host plants, survival time of *H. zea* on both vegetative and reproductive tissues differed significantly in the order of clover>cotton>soybean. Survival time on cotton of HzNPV-infected *H. virescens* fed on vegetative tissues was also significantly higher than on reproductive tissues. Across host plants, survival time of larvae on vegetative tissues differed significantly in the order of cotton>soybean>clover. *Helicoverpa zea* or *H. virescens* larvae fed on vegetative tissues produced significantly more OBs than those larvae fed on reproductive tissues of cotton and soybean. *Helicoverpa zea* larvae fed on reproductive tissues of cotton produced significantly less OBs than those fed on reproductive tissues of soybean or clover. OBs produced by *H. virescens* fed vegetative tissues of host plants also differed significantly in the order of soybean>clover>cotton. $LC_{50}$ values for *H. virescens* larvae fed HzNPV were higher when the virus was produced in larvae fed on reproductive tissues than on vegetative tissues on all host plants. OBs produced by *H. zea* or *H. virescens* larvae fed on reproductive tissues were less infectious as compared to OBs produced on vegetative tissues in all host plants, except *H. zea* on cotton. 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, survival time, viral production and viral infectivity in heliothine larvae were affected by both tissue and plant species.

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 2:1196-1199 (1999)
National Cotton Council, Memphis TN

## Introduction

The ability of host plants to modify disease in insect herbivores has been widely documented for the past several decades (Duffey et al. 1995, and references therein). In our model system of host plant species (cotton, crimson clover, soybean, tomato, velvetleaf, (Carolina geranium), insect species (*H. virescens* and *Helicoverpa zea*) and baculovirus (*H. zea* nucleopolyhedrovirus =HzNPV), we have demonstrated that the host plant exerts a highly significant effect upon disease susceptibility (Forschler et al. 1992, Ali et al. 1998). The studies cited above indicate that the host plant may be a critical factor in determining the ability of baculoviruses to regulate the population dynamics of these insects. The scope of investigation has been limited to one aspect of the infection process (i.e., infectivity) and other important factors that determine the epizootic potential of the pathogen (e.g., viral progeny production) were not taken into account.Although we have gained considerable understanding of how host plants may modify the infectivity of viruses, our knowledge of how host plants affect viral persistence, viral production and subsequent infectivity is exceptionally meager (Duffey et al. 1995). Thus, the ability to predict the impact of host plants on the success of viruses in regulating herbivore population dynamics is seriously limited.

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

## Materials and Methods

### Survival Time of Larvae on HzNPV

Larvae to be used in the test were reared to the second instar either on vegetative (leaves) or reproductive (squares for cotton and petals for soybean and clover) tissues to the second instar in 30-cm clear plastic cups containing a layer of 4% agar water (agar cup). Disks were cut from the vegetative and reproductive tissues of the respective host plant and placed on a layer of 4% agar-water to create 25 individual cells for the experiment. A 0.1µl of HzNPV (Elcar) suspension in 0.1% Triton X-100 was applied to each disk. Dosages were approximate $LC_{50}$s; 10, 30 and 100 OBs/larva for clover, soybean and cotton, respectively. 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 disk for each replicate were individually transferred into 30-mm clear plastic agar cups containing either vegetative or reproductive tissues of the respective host and reared for 10 days. The tissues in cups were replaced with fresh tissues on alternate days. Larval

survival was recorded daily. Each treatment was replicated four times.

**Bioassays**

The HzNPV polyhedra in infected larvae were counted (10 larvae per treatment) under a microscope using an improved Neubauer hemacytometer. Serial doses of the viral preparation ($1 \times 10^3$, $3.3 \times 10^3$, $1 \times 10^4$ and $3.3 \times 10^4$ OBs/ml) in distilled water were spread uniformly over the diet surface and air dried. Individual neonates of *H. zea* or *H. virescens* were placed in these cups and reared at $29.5 \pm 1.0^0$ C for 14 d or until pupation. For each concentration 25 larvae were tested. Larval survival was recorded on alternate days.

**Data Analyses**

Survival time of the virus-infected larvae was calculated (Cox and Oakes, 1984, Collett, 1994). Inter-specific (among the host plants) and intra-specific (between the tissues of each host plant) difference in mean survival time of HzNPV-infected *H. zea* and *H. virescens* larvae were determined using Duncan Multiple Range Test and T-Tests procedures, respectively (ProStat). The effect of host plants and stages on survival time of each insect species was analyzed using Cox's proportional hazard model using JMP software (SAS system). The significance of host plant intra-specific (vegetative and reproductive stages) and inter-specific (cotton, soybean and clover) variation in OBs production by larvae was analyzed following Analysis of Variance and GLM Procedures (SAS system). Bioassay results of OB infectivity were used to determine $LC_{50}$s using the Probit Procedures (SAS system).

## Results

**Survival Time of HzNPV-Infected Larvae**

The mean survival times of HzNPV-infected *H. zea* larvae fed on vegetative or reproductive tissues of cotton, soybean and clover ranged from 3.6 to 4.4 d and differed little, if any, with tissue type. For both tissue types, survival time of *H. zea* larvae differed significantly ($P<0.05$) with host plant and were in the order of clover>cotton>soybean (Figure 1). Cox's proportional hazard test showed that the survival time of larvae across tissues was significantly ($P<0.05$) lower on soybean than larvae fed either clover or cotton, which did not differ significantly.

The mean survival times of HzNPV-infected *H. virescens* larvae ranged from 4.2 to 5.1 d. Survival was longer when larvae fed on vegetative rather than reproductive tissues of cotton, but on soybean and clover, tissues did not differ significantly with stage of host. Among the vegetative tissues of host plants, survival times of larvae differed significantly ($P<0.05$) with cotton >soybean >clover. Cox's proportional hazard test showed that across tissue types, the survival time of larvae fed on clover was greater than on either cotton or soybean, which did not differ significantly. A significant interaction occurred among the tissues and host plants of infected *H. virescens* ($P<0.05$) (Figure 1).

**OBs Produced by HzNPV-Treated Larvae**

The mean number of OBs produced by HzNPV-infected *H. zea* larvae ranged from $26.5 \times 10^6$ to $84.0 \times 10^6$ Obs/larva. Production of OBs on cotton and soybean were significantly ($P<0.05$) higher on vegetative than on reproductive tissues, but on clover it did not differ significantly with tissue type. The number of OBs produced by larvae on reproductive tissues of cotton was significantly ($P<0.05$) lower than the number when larvae were fed on soybean or clover, but the latter did not differ significantly (Figure 2).

The mean number of OBs produced by *H. virescens* larvae ranged from $9.1 \times 10^6$/ OBs/larva on reproductive tissues of cotton to $173.7 \times 10^6$ OBs/larva on vegetative tissues of soybean. 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 the number produced by clover did not differ with tissue type. Larvae fed on vegetative tissue of all host plants differed significantly ($P<0.05$) with the number of OBs on soybean>clover>cotton (Figure 2).

There were significant interactions between two insect species, and tissue of host plants, and plants*insect*tissues in terms of OB production. Total number of OBs produced by *H. zea* larvae fed on both vegetative and reproductive tissues of all host plants were significantly ($P<0.05$) higher than the OBs produced by *H. virescens* larvae fed on the same host plants. The total number of OBs produced by *H. zea* and *H. virescens* larvae on both vegetative and reproductive tissues of soybean were significantly ($P<0.05$) higher than on clover or cotton, while those OBs on clover were significantly ($P<0.05$) higher than cotton only. Total number of OBs produced by *H. zea* or *H. virescens* larvae fed on vegetative tissues also were significantly ($P<0.05$) higher than the OBs produced on reproductive tissues of all host plants. Number of OBs produced by *H. zea* or *H. virescens* larvae fed on vegetative and reproductive tissues of all host plants and the respective survival time showed a positive association but the linear relationship was not significantly correlated.

**Bioassays of OBs Produced by HzNPV-Infected Larvae**

The mean $LC_{50}$s for HzNPV produced in *H. zea* treated as second instar ranged from 0.2 OBs/mm$^2$ for larvae fed on vegetative or reproductive tissues of clover to 2.2 OBs/mm$^2$ for larvae fed on reproductive tissues of soybean. However, the $LC_{50}$s of OBs produced in treated larvae did not differ significantly between tissue types for any of the plant species studied. Across plant types, the $LC_{50}$s of OBs produced in *H. zea* larvae fed clover were significantly ($P<0.05$) less than that in soybean and vegetative tissues in cotton (Table 1). The mean $LC_{50}$s of OBs produced by *H.*

*zea* fed vegetative and reproductive tissues of all host plants were significantly ($P<0.05$) negatively correlated ($r^2=0.70$, a=4.59, b= -0.13) with their survival times. Therefore, it appeared that the longer the *H. zea* larvae survived the more infectious the HzNPV they produced.

The $LC_{50}$s for HzNPV produced in *H. virescens* treated as second instar ranged from 0.8 OBs/$mm^2$ for larvae fed on vegetative tissues of clover to 6.3 OBs/$mm^2$ for larvae fed on reproductive tissues of cotton. The $LC_{50}$s for HzNPV from *H. virescens* larvae fed reproductive tissues in comparison with vegetative tissues were over 2, 2 and 4 fold higher and significantly ($P<0.05$) greater in clover, soybean and cotton, respectively. The $LC_{50}$s of HzNPV produced in larvae fed reproductive tissues of cotton were significantly greater than in clover, but the number produced in soybean did not differ significantly from either cotton or 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 was significantly lower than that of soybean. The mean $LC_{50}$s of OBs from the cadavers of *H. virescens* fed vegetative and reproductive tissues from all host plants also suggested a negative relationship with their survival times, but this was not significant ($r^2=0.13$, a=4.72, b= -0.07) (Table 1).

**Total Activity Produced Per HzNPV-Infected Larva ($LC_{50}$ Units)**

The total number of $LC_{50}$ activity units of HzNPV produced by *H. virescens* larvae were higher when fed vegetative than reproductive tissues of all the host plants. HzNPV-infected *H. virescens* larvae fed reproductive tissues of clover, cotton and soybean, respectively, yielded 66.9, 91.5 and 91.5% less $LC_{50}$ activity units of the virus than larvae fed vegetative tissues. Within a tissue the total $LC_{50}$s produced in either tissue for *H. virescens* were greater in soybean or clover than cotton. The number of $LC_{50}$ activity units of HzNPV produced in *H. zea* on soybean was higher when fed vegetative rather reproductive tissues, but did not differ with tissue type on the other host plants. In addition, the total number of $LC_{50}$s produced in *H. zea* on clover in both tissue types was higher than in cotton or soybean (Table 1).

## Discussion

The host plant exerts a profound influence upon the epizootic potential of HzSNPV. Previously investigators have emphasized the direct effects of host plants on viral infectivity (Duffey et al. 1995), but here we report that large variations in both viral production and subsequent viral infectivity were found depending upon the host plant of the insect. When the total activity produced (=number of OBs / $LC_{50}$) is considered, the influence of the host plant is striking. A 16-fold difference occurs in *H. zea* among hosts while in *H. virescens* more than a 75-fold difference is found. Our findings indicate that the influence of the host plant may have been greatly underestimated in previous studies.

In most instances, the infectivity and numbers of OBs produced is greatest with clover as a host compared to cotton and soybean for both insect pests. Clover is one of several early season host plants of heliothines (Stadelbacher 1979 1981, Hendricks 1992) and grows abundantly near roadsides throughout the Mississippi River Delta. These hosts provide refuge for first generation heliothine larvae produced by overwintering pupae. The importance of suppressing the early season generations has been emphasized for area-wide management of heliothines utilizing aerial applications of HzSNPV (Stadelbacher 1979 1981, Mueller et al. 1984, Hendricks 1992). Based upon our current findings and those reported earlier (Ali et al., 1998), applications of HzSNPV in clover appear to offer the most potential for producing epizootics.

Our results revealed that the number of OBs produced by *H. zea* or *H. virescens* larvae fed vegetative tissues were significantly ($P<0.05$) higher than those fed on reproductive tissues in all tested plant species, except on clover. We previously showed that larval susceptibility to HzSNPV was generally decreased when feeding on reproductive tissues of most hosts (Ali et al.,1998). These findings illustrate the difficulty in controlling heliothines due to their propensity to feed on reproductive structures of their hosts, where they not only avoid mortality factors such as predators, parasitoids, insecticides and adverse environmental conditions, but also minimize their risk of infection.

Viral inactivation by sunlight has been long recognized as a nearly insurmountable barrier to the effective deployment of viral pesticides; however, our data strongly support the notion that the host plant is also a formidable barrier to the establishment and spread of baculovirus in an insect population. Not only does the ingestion of certain host plants (e.g., cotton) severely limit the establishment of lethal infections in heliothine larvae, but it also restricts the production of viral occlusion bodies and their subsequent infectivity to other intra-/intergenerational larvae. Moreover, the persistence of occlusion bodies is compromised on leaf surfaces of certain plants such as cotton (Doug Streett et al., unpublished data, Young and Yearian 1974, Elleman and Entwhistle 1985). A greater emphasis on mechanistic studies of how host plants influence baculoviral disease is essential to develop strategies that may circumvent the host plant barrier and facilitate more effective utilization of viral pesticides (Duffey et al. 1995).

## References

Ali, M. I., Felton, G. W., Meade, T., and Young, S. Y. 1998. Influence of host inter-specific and intra-specific variation on the susceptibility of heliothines to a baculovirus. Biologic. Contr. 12: 42-49.

Ali, M. I., Young, S. Y., and Yearian, W. C. 1985. Transmission of a nuclear polyhedrosis virus in *Heliothis zea* (Lep.: Noctuidae) larval populations on soybean. Entomophaga 30: 167-175.

Alvarez, C. S., and Garcia, R. O. 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.

Anderson, R. M. and May, R. M. 1981. The population dynamics of microparasites and their invertebrate hosts. Phil. Trans. Royal Soc. London, Series B, 291: 452-524.

Appel, H.M. 1994. The chewing herbivore gut lumen: Physiological conditions and their impact on plant nutrients, allelochemicals, and insect pathogens, pp. 209-223, in E. A. Bernays (ed.). Insect Plant Interactions. CRC Press, Boca Raton, Fl.

Barber, G. W. 1941. Hibernation of corn earworm in Southeastern Georgia. Technic. Bull., *USDA*, , 791,1-17.

Boucias, D. G., Johnson, D. W., and Allen, G. E. 1980. Effects of host age, virus dosage, and temperature on the infectivity of nucleopolyhedrosis virus against velvetbean caterpillar, *Anticarsia gemmatalis*, larvae. Environ. Entomol. 9: 59-61.

Collett, D. 1994. Modeling survival data in medical research. Chapman and Hall. London 347 pp.

Cox, D. R., and Oaks, D. 1984. Analysis of survival data. Chapman and Hall. London 201 pp.

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

Elleman, C. J. and Entwistle, P. F. 1985. Inactivation of nuclear polyhedrosis virus on cotton by substances produced by the cotton leaf glands. Ann. Appl. Biol. 106: 83-92.

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

Foster, M. A., Schultz, J. C. and Hunter, M. D. 1992. Modelling gypsy moth-virus-leaf chemistry interactions: implications of plant quality for pest and pathogen dynamics. J. Animal Ecol. 61: 509-520.

Hendricks, D. E. 1992. The role of velvetleaf growing in or near cotton fields as a host *for* tobacco budworms and bollworms in the Mississippi Delta. Southwestern Entomol. 17: 199-207.

Mueller, T. F., Harris, V. E., and Phillips, J. R. 1984. Theory of *Heliothis* (Lepidoptera: Noctuidae) management through reduction of the first spring generation. A critique. Environ. Entomol. 13: 625-634.

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: 153-1156.

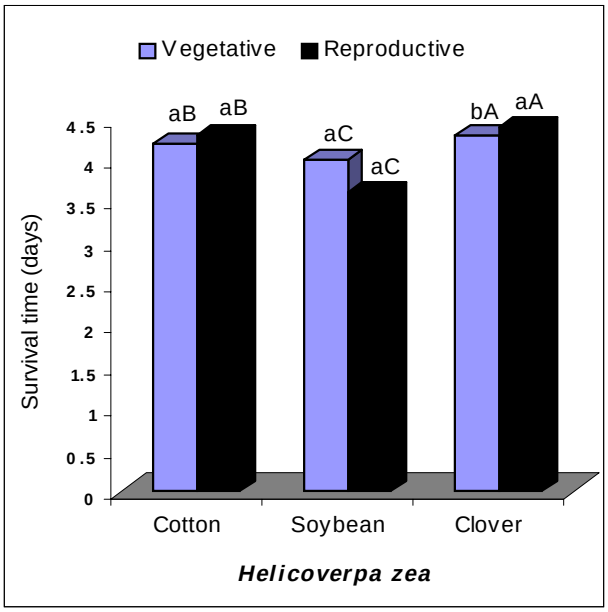
Stadelbacher, E. A. 1981. Role of early-season wild and naturalized host plants in the buildup of the $F_1$ generation of *Heliothis zea* and *H. virescens* in the Delta Mississippi. Environ. Entomol. 10: 776-770.

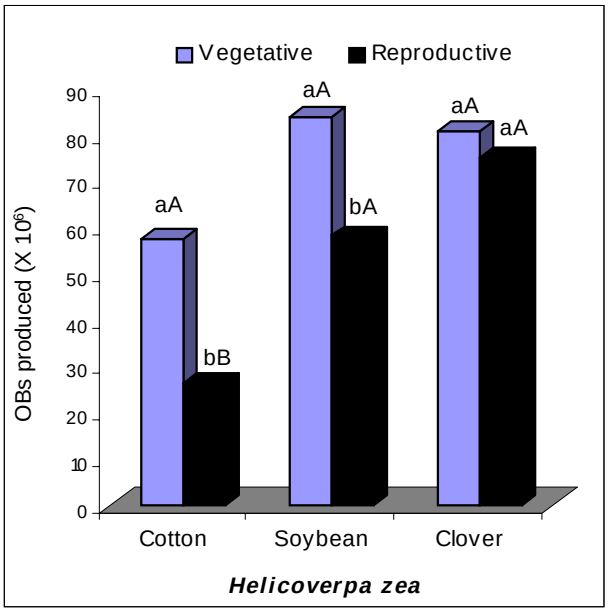
Young, S. Y., and Yearian, W. C. 1974. Persistence of *Heliothis* NPV on foliage of *cotton*, soybean and tomato. Environ. Entomol. 3: 253-255.

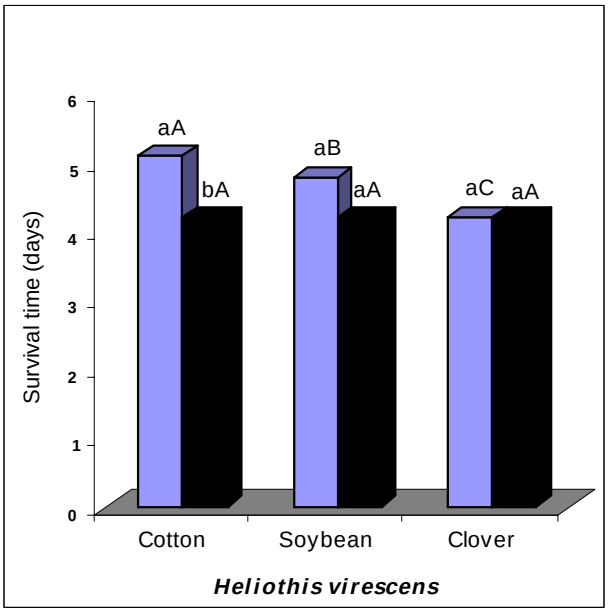
Table 1. $LC_{50}$s and total activity of OBs produced by heliothine larvae fed on veg. or rep. tissues of different host plants.

| Plant/tissue | $LC_{50}$ Obs/mm[a,b] (95% Fiducial limit) | Slope | Total activity produced/ larva in $LC_{50}$ units (±SE)[2] |
|---|---|---|---|
| | *Bollworm* | | |
| Cotton | | | |
| Vegetative | 1.3 (0.7-3.2) | 1.3 | 44.4 (5.3) X $10^6$ |
| Reproductive | 0.5 (0.2-0.9) | 1.5 | 56.4 (12.7) X $10^6$ |
| Soybean | | | |
| Vegetative | 1.0 (0.8-1.1) | 1.2 | 88.4 (9.6) X $10^6$ |
| Reproductive | 2.2 (0.9-22.9) | 1.0 | 27.0 (1.6) X $10^6$ |
| Clover | | | |
| Vegetative | 0.2 (0.2-0.2) | 1.8 | 450.0 (74.7) X $10^6$ |
| Reproductive | 0.2 (0.2-0.2) | 1.7 | 395.7 (57.9) X $10^6$ |
| | *Budworm* | | |
| Cotton | | | |
| Vegetative | 1.6 (0.7-9.0) | 0.9 | 16.6 (4.5) X $10^6$ |
| Reproductive | 6.3 (3.4-19.6) | 0.7 | 1.5 (0.3) X $10^6$ |
| Soybean | | | |
| Vegetative | 1.5 (1.2-2.0) | 0.9 | 113.6 (8.8) X $10^6$ |
| Reproductive | 2.9 (2.1-4.8) | 1.0 | 9.7 (8.8) X $10^6$ |
| Clover | | | |
| Vegetative | 0.8 (0.6-1.0) | 0.7 | 71.6 (9.4) X $10^6$ |
| Reproductive | 1.6 (1.3-2.2) | 0.8 | 23.7 (3.9) X $10^6$ |

[a] Total activity per larva was calculated by dividing OBs produced per larva (Table 3) by the respective $LC_{50}$ (OBs/$mm^2$ of diet surface).
[b] Mean number of larvae posttreatment = 1025-1125, except for Budworm cotton and soybean rep. plant the number is 525.



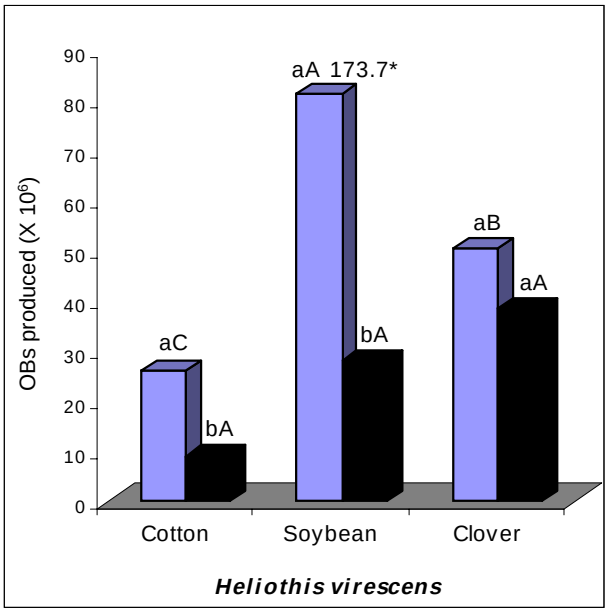




Figure 1: Survival time of Hznpv-treated second instar heliothines on different host plants. Means in a crop (lower case) or in a color among crops (upper case), followed by same letter(s) are not significantly different (P > 0.05, by Paired T-Test).

Figure 2: Number of Occlusion Bodies (Obs) produced by a second instar Hznpv-treated heliothine on different host plants. Means in a crop (lower case) or in a color among crops (upper case), followed by same letter(s) are not significantly different (P > 0.05, by Paired T-Test). * number of Obs = 173.7 X $10^6$.