Influence of Agronomic Hosts on the Susceptibility of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctidae) to Genetically Modified (Bollgard®) and Conventional Cotton Plants

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**Abstract**

In present study bioassays conducted on *Helicoverpa armigera* neonates (F₂). Pupae surviving from each host specific colony were maintained as previously described. Egg sheets were harvested daily and placed into plastic bags. Upon eclosion, 200 F₂ *Helicoverpa armigera* neonates were offered leaves (<5 cm) harvested from Bollgard® and conventional cotton plant terminals and held in petri dishes. Treatment was arranged in a randomized complete block design where d of larval eclosion constituted blocks. All *Helicoverpa armigera* developmental stages were maintained at 27 ± 2°C and 85 ± 5% relative humidity. *Helicoverpa armigera* larval mortality on Bollgard® and conventional cotton plants was compared among the different host specific colonies 96 hrs after exposure to cotton tissue. Analysis of variance (ANOVA) was made by using IBM® SPSS, also means were separated according to Tukey’s Studentized Range Test.

**INTRODUCTION**

For any pest management system to be effective, we need knowledge of the population dynamics of the target pests in relation to their various host plants (Fitt, 1989 and Dent, 1991). Polyphagy is considered a key component of *Helicoverpa* population dynamics and pest status (Fitt, 1989). *Helicoverpa armigera* (Hubner) exploits multiple hosts concurrently or in succession. *Helicoverpa armigera* is unique in that it has three common names accepted by the Entomological Society of India, depending on the host plant. This insect is also feed on corn earworm on corn, *Zea mays* L.; the tomato fruit worm on tomato, *Lycopersicon esculentum* (Miller); and the bollworm on cotton plants, *Gossypium hirsutum* L. Larvae have been reported on more than 100 wild and cultivated plant hosts.

In addition to corn and cotton plant, *Helicoverpa armigera* also feeds on soybean, *Glycine max* L., and grain sorghum, *Sorghum bicolour* (L.). *Helicoverpa armigera* population densities on these hosts are usually not as numerous as those found in cotton plant. However, *Helicoverpa armigera* can be an annual pest of field corn, cotton plant, soybean, and grain sorghum in India. Multiple integrated pest management tactics are used to prevent *Helicoverpa armigera* from reaching damaging levels; however, insecticides have been the primary tool used in most production systems. *Helicoverpa armigera* development on selected host plants has been studied extensively.
Gross and Young (1977) determined the period from larval eclosion to pupation of Helicoverpa larvae on field corn, various non-cultivated hosts, and a meridic diet. Larvae required a longer period to develop on corn foliage (30.6 d) compared to the meridic diet (21.8d) at day: night: temperatures of 26:15°C. Also, pupal weights were lower on corn foliage (268 mg/insect) compared to meridic diet (447 mg/insect) (Gross and Young, 1977). Based on the ability of Helicoverpa armigera larvae to complete development under field conditions, field corn was a better host than cotton plant or grain sorghum (Harding, 1976). Hayes (1988) released adults into field cages that contained various host plants and determined that Helicoverpa armigera developed faster on grain sorghum compared to larvae that developed on cotton plant and corn. Although some variation occurred in the results of these studies, the investigators rated host suitability based on a single factor rather than all of the factors that affect insect performance. Also, no information was presented about plant developmental stages. Hartstack et al., (1973) and Roach and Ray, (1976) determined that the density of Helicoverpa adults produced on field corn was higher than on other agronomic crops. Sparks et al., (1971) study shows that, Helicoverpa larvae were introduced into field cages over corn; tobacco, Nicotiana tabacum L.; cotton; sesame, Sesamum indicum L.; and soybean. Field corn produced more pupae than cotton plant or the other hosts.

Little information is available about Helicoverpa populations from various host plants and their subsequent development on cotton plants. This information will be important to effectively integrate genetically engineered Bollgard® cotton plant into current pest management systems. Bollgard® cotton plant was developed by incorporating a foreign gene from Bacillus thuringiensis, into cotton plants (Perlak et al., 1990). These Genetically Modified cotton plants produce the cryIAc protein from Bacillus thuringiensis which is selectively toxic to the larval stages of several Lepidopteran insects (MacIntosh et al., 1990, Luttrell et al., 1999). Although the primary targets were tobacco budworm, Helicoverpa virescens (F.), and pink bollworm, Pectinophora gossypiella (Saunders), also Helicoverpa armigera are susceptible to the cryIAc protein. However, Bollgard® cotton plant has not provided satisfactory control of Helicoverpa armigera under certain situations. Agencies, industry, producers, and academic researchers are concerned with the development of resistance to Bollgard® cotton plants and have adopted resistance management plans for target pests (Gould and Tabashnik, 1998). These plans rely on the use of refuges (Gould, 1998) to produce susceptible populations. Initial plans were developed for pests with narrow host ranges such as Helicoverpa virescens and Pectinophora gossypiella and the contribution of alternate hosts for the production of susceptible populations of these species has not been extensively considered. This is primarily due to the fact that Pectinophora gossypiella feeds only on cotton plant and Helicoverpa virescens has a relatively limited host range in most areas of the United States. Alternate hosts may effectively serve as refugia for the production of susceptible populations of polyphagous insects such as Helicoverpa zea (Fitt, 1989). However, before the role of alternate hosts can be evaluated as refugia, information on Helicoverpa armigera development on agronomic crops such as field corn, soybean, and grain sorghum in areas adjacent to cotton plant should be determined. Also, the survival of subsequent Helicoverpa armigera generations on Conventional and Bollgard® cotton plant from populations surviving on those hosts needs to be evaluated. These studies examine Helicoverpa armigera performance on selected agronomic hosts and the influence of those hosts on subsequent Helicoverpa armigera survival on non-Bollgard and Bollgard® cotton plant.

MATERIALS AND METHODS
A Helicoverpa armigera colony was established from sweet corn and maintained on meridic diet for one generation in the laboratory. Approximately 200 to 300 larvae (third instar) were collected daily from sweet corn ears. Larvae were placed in 29.5 ml plastic cups with a soy protein/wheat germ based meridic diet and transported to the laboratory. Moths were placed in 3.8 L cardboard containers and fed a 10% sucrose solution. A single layer of cheesecloth was placed on the top of buckets for moth oviposition. Oviposition sheets were harvested daily and placed into 118 x 59 x 354 cm plastic bags. Larvae eclosing from these eggs were separated into five host specific colonies and utilized for bioassays.
Development of *Helicoverpa armigera* Host Colonies

Plots of conventional cotton plant (cv Rasi 2000), field corn, grain sorghum, and soybean were planted at the Village Vihamandwa of Paithan talsil of Aurangabad district. Plots consisted of four 9.1m rows and included one row each of cotton plant, field corn, grain sorghum, and soybean. Crop hosts were planted on multiple dates (i.e. 14, 21 and 28 June 2013) to ensure that the plant stages preferred by *Helicoverpa armigera* were available at the proper timing. Neonate *Helicoverpa armigera* (F₁) from the field-collected colony were offered tissues from cotton plant, soybean, field corn, or grain sorghum or a meridic diet in individual 29.5 ml plastic cups until pupation. *Helicoverpa armigera* reared on cotton plant were presented with flower buds (squares, 10 to 15 mm diameter) removed from plants at the nodes above white flower.

Larvae reared on grain sorghum were offered pieces of seed heads in the soft-dough stage (Vanderlip, 1993) throughout larval development. A separate control colony also was maintained on the meridic diet used for the original collection from sweet corn. Two separate cohorts of insects were maintained on each host and meridic diet. Cohorts served as blocks in a randomized complete block design and were initiated with 1000 neonates for each host. Plant tissue was changed every 48hr until pupation. Meridic diet (ca. 8g) was not changed throughout the duration of larval development. Larval survival, time to pupation, and pupal weights for each host and meridic diet were recorded. Data was analyzed with analysis of variance (ANOVA).

Mortality of *Helicoverpa armigera* on Bollgard® and Conventional Cotton Plants

Plots of Bollgard® and conventional cotton plants were planted on 11 June 2013 for bioassays conducted on *Helicoverpa armigera* neonates (F₃). Pupaes surviving from each host specific colony were maintained as previously described. Egg sheets were harvested daily and placed into plastic bags. Upon eclosion, 200 F₂ *Helicoverpa armigera* 90 neonates were offered leaves (<5 cm) harvested from Bollgard or conventional cotton plant terminals and held in petri dishes. Treatment was arranged in a randomized complete block design where d of larval eclosion constituted blocks. All *Helicoverpa armigera* developmental stages were maintained at 27 ± 2°C and 85 ± 5% relative humidity. *Helicoverpa armigera* larval mortality on Bollgard® and conventional cotton plants was compared among the different host specific colonies 96 h after exposure to cotton tissue. Analysis of variance (ANOVA) was made by using IBM® SPSS, also means were separated according to Tukey’s Studentized Range Test (Tukey, 1977).

RESULTS AND DISCUSSION
Development of *Helicoverpa armigera* Host Colonies

*Helicoverpa armigera* survival varied among diets ($F_{3,3} = 35.04; df=3, 3; P<0.01$). Survival declined to less than 65% within 2 days on soybean and cotton plant. Initial *Helicoverpa armigera* survival remained relatively high (>90%) on field corn, grain sorghum, and meridic diet. However, survival declined to less than 72% within 8 day on field corn. At 16 day, *Helicoverpa armigera* survival declined to less than 83% on grain sorghum. Survival remained greater than 85% on meridic diet. Total *Helicoverpa armigera* survival was higher on meridic diet (85%) and grain sorghum (70%) than survival on soybean (28%), and cotton (15%). Also, *Helicoverpa armigera* survival on field corn (58%) was higher than survival on cotton plant (Figure 1). Differences in survival of F₁ larvae among the host specific colonies may have been due to variations in levels of nutrients and/or plant secondary compounds. Meridic diets are developed to provide optimum nutrition with minimal amounts of toxic substances. In contrast, many plant species produce specific allelochemicals such as tannins, phenolics, and terpenoids, which may adversely affect insect development. Cotton, field corn, soybean, and grain sorghum plants produce numerous allelochemicals that adversely affect insect development and survival. *Helicoverpa armigera* from the various host plant and meridic diet colonies varied in their times to completion of all larval stadia. Intervals to pupation were different among host plants and meridic diet ($F_{3,4} = 34.50; df=4, 4; P<0.01$). *Helicoverpa armigera* completed larval stadia more rapidly on field corn (12 day) than all other host plants or meridic diet (Figure 2). Completion of larval stadia for *Helicoverpa armigera* offered soybean (18 day) was shorter than for *Helicoverpa armigera* offered grain sorghum (15 day), cotton (25 day), or meridic diet (15 day) (Figure 3). *Helicoverpa armigera* took longer to complete larval stadia on cotton than all other plant hosts.
In addition, all larvae achieved the pupal stage over a range of 5 day, 6 day, 8 day, 7 day, and 8 day on field corn, meridic diet, grain sorghum, soybean, and cotton, respectively. In a similar study, Helicoverpa armigera development from larval eclosion to pupation required 30 day on corn foliage (Gross and Young, 1977). This is considerably longer than observations in the present study (12 day) but their experiment was conducted at lower night temperatures. Also, Helicoverpa armigera larvae prefer to feed on structures that contain high levels of nitrogen (i.e. reproductive structures) (Fitt, 1989). Corn seed may have provided a higher level of nutrition for Helicoverpa armigera larvae than foliage; therefore, larvae would be expected to develop faster on seed than foliage. In our study, Helicoverpa armigera larvae fed cotton required 25 day to pupate (Figure 4). The sesquiterpene gossypol, an allelochemical found in cotton, delays development and reduces larval weight of Helicoverpa spp. (Hedin et al., 1983). Consequently, survival and pupal weights were lowest on cotton compared to the other hosts. Also, larval developmental time was longer on cotton than the other hosts. Pupal weights were higher for larvae reared on meridic diet (35.5 mg) compared to larvae fed cultivated host plants \(F=62.05; df=4, 4; P<0.01\). Pupal weights on cotton, corn, grain sorghum, and soybean averaged 235, 290, 310, and 340 mg, respectively. Weights of pupae from larvae fed cotton were lower than pupal weights on all other larval diets.

Mortality of Helicoverpa armigera Host Colonies on Conventional and Genetically Modified (Bollgard®) Cotton

In present study, observation on Helicoverpa armigera larval diet influenced the mortality of subsequent generations on cotton plant. Mortality on non-Bollgard cotton plant was different among \(F_2\) Helicoverpa armigera larvae from the host colonies \(F=4.60; df=4, 12; P=0.02\). Helicoverpa armigera mortality averaged 52.6, 34.6, 12.0, 10.0, and 7.5% for the cotton plant, grain sorghum, corn, soybean, and meridic diet colonies, respectively.

Helicoverpa armigera mortality on conventional cotton plants was higher for the cotton plant colony than the soybean, field corn, and meridic diet colonies. Bollgard® cotton plant produced variable levels of mortality among \(F_2\) Helicoverpa armigera larvae from the host colonies \(F=4.60; df=4, 12; P=0.02\). Mortality of Helicoverpa armigera from the cotton, grain sorghum, field corn, soybean, and meridic diet colonies averaged 74, 60, 80, 62, and 70%, respectively, on Bollgard® cotton plant. Helicoverpa armigera mortality on Bollgard® cotton plants was higher for the field corn colony than the soybean and grain sorghum plant colonies.

Plant hosts and meridic diets can influence the activity of various mortality factors including insecticides (Berry et al., 1980, Wood et al., 1981, Muehleisen et al., 1989, Tan and Guo 1996), bacteria (Moldenke et al., 1994), nuclear polyhedrosis viruses (Richter et al., 1987; Keating et al., 1988, 1989; Peng et al., 1997), fungi (Hare and Andreidis 1983, Ramoska and Todd 1985), and nematodes (Barbercheck et al., 1995). Multiple factors associated with host plants can influence insect susceptibility to toxic substances. Several studies have documented induction by host plants of detoxifying enzymes in insect pests (Yu 1982, 1984). This may be an important factor in reducing the effects of some toxins, especially synthetic insecticides (Berry et al., 1980, Moldenke et al., 1994). All of the previous studies were conducted during the same generation of insects that were fed the different host plants.

In present study, bioassays with conventional and Bollgard cotton were conducted on the generation \(F_2\) following the one \(F_1\) that was exposed to the different host plants. Therefore, induction of detoxifying enzymes is not a likely cause for differences observed in bollworm Helicoverpa armigera mortality on Bollgard® cotton plant because induction is temporary and non-hereditary. Some individuals within a population may have inherently higher enzyme levels than other individuals. In this instance, those larvae from specific host colonies may have been selected with enzymes that increase insect performance on the different hosts.

The host colonies were not combined and the frequency of individuals with high enzyme levels would have increased if inheritance of that trait was recessive, thereby, resulting in differences in \(F_2\) larval mortality among the different hosts. However, if this were the case in our study, bollworm mortality from the cotton plant colony would be expected to be lower than the other host colonies on both non- Bollgard and Bollgard cotton plant. Nutrition is another factor that may contribute to differences in insect mortality.

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Moldenke et al., (1994) suggested that gypsy moth larvae fed alder, Alnus rhombifolia Nuttall, may have been less susceptible to Bacillus thuringiensis than larvae fed Douglas fir, Pseudotsuga menziesii Franco, because higher levels of nitrogen were available in alder. Differences in fall armyworm (Richter et al., 1987) and velvetbean caterpillar, Anticarsia gemmatalis Hubner, (Peng et al., 1997). Barber (1936,) studied susceptibility to nuclear polyhedrosis viruses can be attributed to host suitability. Field corn is a preferred host for Helicoverpa armigera development Helicoverpa armigera developed similarly on field corn, grain sorghum, and meric diet. In contrast, F1 larval survival, developmental time, and pupal weights were poor on cotton plant compared with the other hosts. Lukefahr and Martin (1964) determined that adult fecundity was influenced by larval diet. Moths that were fed cotton plant during the larval stage did not produce viable eggs when fed water alone during the adult stage. In contrast, 72.8 to 88.0% of eggs laid by moths from larvae that fed on corn or meric diet were viable when the moths were fed only water. It shows that corn and meric diet were sufficient to produce viable eggs without the adults receiving additional nutrition. Cotton plant was not sufficient as a larval diet for subsequent adults to produce viable eggs unless they were provided a sugar water solution.

Previous host plants in the current study may have influenced survival of Helicoverpa armigera on Bollgard cotton plant based on their relative nutritional value for F1 larvae. Agronomic crops other than cotton plant provide a source of Helicoverpa armigera during much of the season in the southeastern and mid-southern United States. Based on the combination of all developmental factors from our study as well as data from other studies, field corn appears to be the most suitable host plant for Helicoverpa armigera. During the period when corn is most susceptible to Helicoverpa armigera feeding, few larvae are present in cotton plant. Therefore, field corn may not provide a source of Helicoverpa armigera adults at the proper time of year to mate with Helicoverpa armigera adults emerging from Bollgard cotton plant. Consequently, when Helicoverpa armigera populations peak in cotton plant, field corn is no longer attractive. However, large numbers of Helicoverpa armigera develop on corn and this may effectively dilute resistance alleles from the previous season before Helicoverpa armigera moves into cotton plant. Helicoverpa armigera moths will oviposit on soybean foliage (Hillhouse and Pitre 1976, Pitre and Hillhouse 1981) and grain sorghum seed heads (Cronholm et al., 1998) during the flowering stages of 100 each of these hosts.

The flowering stages of these hosts correspond with the preferred ovipositional stages of cotton plant in the southern United States (Johnson et al., 1975). Population densities on soybean and grain sorghum are generally lower than that observed on cotton plant, but a higher percentage of larvae develop to pupation on these hosts compared to cotton plant. Because Helicoverpa armigera survival on soybean and grain sorghum is higher than on cotton plant and they are present at the same time on cotton plant, these hosts may provide a source of Helicoverpa armigera adults to mate with moths emerging from Bollgard cotton plant. In a similar study, Losey et al., (2001) determined that alternative hosts not support European corn borer, Ostrinia nubilalis (Hubner), densities at a sufficient level to contribute to a resistance management plan for Bt-corn. However, in that study the alternative hosts were not as attractive as corn for oviposition and larval survival was lower on the other hosts than on corn.

In contrast, previous studies indicate that Helicoverpa armigera oviposition is similar among various hosts depending on the host’s growth stage (Stadelbacher, 1980). Also, based on the present study, more Helicoverpa armigera larvae survived on the alternate hosts evaluated than on cotton plant. Similarly, velvetleaf, Abutilon theophrasti (L.), could support sufficient populations of Helicoverpa armigera and Helicoverpa virescens for consideration as a refuge for Bollgard cotton plant. The role of the major cultivated host plants in the mid-southern United States on Helicoverpa armigera population densities and their relationship to cotton plant should not be underestimated in the design/implementation of integrated pest management and resistance management strategies. Field corn produces large numbers of Helicoverpa armigera that subsequently serve as a source of initial populations that migrate into cotton plant during late June and early July.

A study conducted during 1964 over a 27 square mile area in Arkansas determined that Helicoverpa armigera populations achieve high densities on field corn during mid- to late-June and early July.
Subsequent *Helicoverpa armigera* populations were observed at varying densities on grain sorghum, soybean, and cotton plant during July and August. Grain sorghum and soybean may serve as a source of *Helicoverpa armigera* re-infestations during July and August after applications of foliar insecticides have reduced populations in cotton plant. However, before these crops can be considered for refuges in a resistance management strategy, studies need to be conducted to determine specific numbers of *Helicoverpa armigera* adults contributed by each of these hosts under field conditions.

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**REFERENCES**


**Environmental Protection Agency.** 2001. *Bt* cotton refuge requirements for the 2001 growing season.


**Gross, HR Jr., JR Young**, 1977. Comparative development and fecundity of corn earworm reared on selected wild and cultivated early-season hosts.


Keating ST, WG Yendol, JC Schultz, 1988. Relationship between susceptibility of gypsy moth larvae (Lymantriidae) to a baculovirus and host plant foliage constituents. Env. Entomol. 17: 952-958.

Keating ST, WJ McCarthy, WG Yendol, 1989. Gypsy moth (Lymantria dispar) larval susceptibility to a baculovirus affected by selected nutrients, hydrogen ions (pH), and plant allelochemicals in artificial diets. J. Invert. Pathol. 54: 165-174.


Moldenke AF, RE Berry, JC Miller, JG Wernz, and XH Li, 1994. Toxity of Bacillus thuringiensis subsp. kurstaki to gypsy moth, Lymantria dispar, fed with alder or Douglas-fir. J. Invert. Pathol. 64: 143-145.


Sparks AN, BR Wiseman, and WW McMillian, 1971. Production of corn earworms on several hosts in field cages. J. Econ. Entomol. 64: 540-541.


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