Effect of larval diets on the survival and development of larvae in the cotton bollworm, *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae)

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(Received 14 June 1999; Accepted 4 November 1999)

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**Abstract**

This study examined the influence of seven natural diets (cotton boll, cotton leaf, okra fruit, soybean seed, tomato fruit, tomato leaf, and corn kernel) and an artificial diet (Insecta LF) on the larval survival rate, larval duration, frequency of larval stadium types and pupal weight in *Helicoverpa armigera*. On most diets, the survival rate was notably lower in the early larval stage compared to the late one. The percentage of larvae that survived from the 1st-stadium to the end of the larval stage was highest on okra fruit and Insecta LF (>60%). Less than 60% of the larvae fed on the other diets completed their larval period. The mean larval period was significantly shorter for individuals reared on plant fruits or seeds as well as on Insecta LF than for those reared on plant leaves. The shortest larval duration was recorded on cotton boll while the longest was seen on cotton leaf. The number of larval stadia ranged from 5 to 6 on cotton boll, soybean seed, corn kernel and Insecta LF, 5 to 7 on okra and tomato fruits, and 6 to 7 on cotton and tomato leaves. The five-stadium type was dominant on cotton boll, okra fruit, corn kernel and Insecta LF, while the 6-stadium type was common on soybean seed, tomato fruit, cotton leaf and tomato leaf. Mean pupal weight was greatest in individuals fed on Insecta LF and least in those fed on tomato leaf.

**Key words**: *Helicoverpa armigera*, larval diet, survival rate, larval duration, larval stadium

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**INTRODUCTION**

The cotton bollworm, *Helicoverpa armigera* Hübner, is a key agricultural pest occurring in many parts of the world including Africa, India, central and southeast Asia, the Middle East, southern Europe, eastern and northern Australia, New Zealand and many eastern Pacific Islands (Pearson, 1958; Fitt, 1989). In Japan, field populations of this species had been endemic until outbreaks occurred in some areas of western Japan in 1994 (Yoshimatsu, 1994).

The pest status of *H. armigera* is due in part to the highly polyphagous nature of its larvae (Fitt, 1989; King, 1994). Worldwide, *H. armigera* has been recorded from at least 60 cultivated and 67 wild host plants (Reed and Pawar, 1982). Extensive surveys undertaken recently in the inland areas of Australia increased the known hosts of this species by 26 (Zalucki et al., 1994), suggesting that the host range is actually wider than what is known. A diverse range of food, fiber, oil, fodder, horticultural and ornamental crops are among the economically important hosts attacked by *H. armigera*.

Although the larvae of *H. armigera* have a considerably wide host range, the rate of larval survival and development greatly varied on different host plants (Pearson, 1958; Coaker, 1959; Zalucki et al., 1986). Nevertheless, polyphagy offers *H. armigera* an avenue for population survival and persistence (Fitt, 1989).

However, in Japanese *H. armigera* strains, the polyphagous nature of its larvae has been scarcely studied. Therefore, we examined the influence of seven natural diets and one artificial diet (Insecta LF) on the larval survival rate, larval duration, frequency of larval stadium types and pupal weight of *H. armigera*.

**MATERIALS AND METHODS**

**Experimental conditions.** The experiment was conducted in a small room (1.70 m in length ×
1.70 m in width × 1.98 m in height) wherein the environmental conditions (16L8D, 25 ± 1°C) were controlled. Five 20-W fluorescent lamps provided illumination during the day while a 60-W light bulb enclosed in an inverted black plastic container with several small holes on top provided background illumination during the night.

**Insects and mass rearing procedure.** The *H. armigera* populations initially collected as late-stadium larvae on cabbage in Ushimado, Okayama Prefecture and on okra in Ishigaki Island, Okinawa Prefecture in late autumn, 1996, were used in this study. The Ushimado population was used in all treatments except on soybean seed where the Okinawa population was used.

The field collected larvae were reared individually in 50-ml glass vials and fed on Insecta LF (Nihon-Nosan Kogyo Co.), a commercially formulated artificial diet. Fresh slices of this diet were provided to the larvae every 4 days. The pre-pupae bored into the diet and pupated. One-week-old pupae were removed from the vials and sorted according to sex on the basis of the configuration of the anal and genital pores. The sorted pupae were subsequently transferred into Petri dishes half-filled with small paper towel strips and put into screen cages (30 cm × 30 cm × 30 cm).

On night 1 (the night following adult eclosion), 8 to 10 pairs of females and males were confined in each screen cage and were fed ad libitum on 10% (w/v) honey solution. Around 3 to 4 pieces of thin cotton sheetings were taped onto the side walls of each screen cage for oviposition by the females. Every day, these egg-containing substrates were removed from the cage, cut into smaller sheets and transferred into Petri dishes and covered. Subsequently, new oviposition sheetings were set in the cages.

Upon egg hatching, small slices of Insecta LF were put into the Petri dishes as larval food. Larvae were individually reared in 50-ml glass vials starting from the late 2nd-stadium. The larval diet was replenished every 4 days and the insects pupated in the diet. The same rearing procedure was followed for every laboratory generation of the insect until the start of the experiment.

**Larval diets.** Five plants, namely, cotton (*Gossypium hirsutum*), okra (*Abelmoscus esculentus*), soybean (*Glycine max*), cherry tomato (*Lycopersicon esculentum*), and sweet corn (*Zea mays*), were established on three planting schedules in two small fields inside the campus of Okayama University in late spring and early summer, 1997. No pesticides were sprayed on the plants. Seven natural diets, i.e., young cotton boll, young cotton leaf, young okra fruit, immature soybean seed, young cherry tomato fruit, young cherry tomato leaf, and immature sweet corn kernel were taken from these plants and used as larval food. For control larvae, the artificial diet Insecta LF was given.

**Procedure of larval rearing on the diets.** Newly hatched 1st-stadium *H. armigera* larvae were individually transferred from the Petri dishes to 50-ml glass vials containing fresh diets using a small, soft brush moistened with distilled water. The numbers of 1st-stadium larvae reared on the diets varied from 54 to 138. For ventilation, a small hole (1.5 mm in diameter) was made in the center of each vial cap by using a hand drill. To prevent the larvae from escaping, a small piece of a fine nylon gauze was placed on the mouth of each vial before the cap was screwed. A circular paper towel was fitted at the bottom of each vial in order to absorb excess moisture. From the 1st- to the 3rd-stadia, the cotton bolls, okra fruits and tomato fruits were sliced into small pieces (4 to 6 slices per fruit) using a kitchen knife, but the fruits of both plants were halved starting from the 4th-stadium as the larvae became larger and more voracious. The soybean seeds and corn kernels were provided to the larvae as half and whole seeds, respectively. Only the young cotton or tomato leaves taken from the active growing parts of the plants were used as diets. Fresh natural diets and the 50-ml glass vials were replenished daily. Small cuts of Insecta LF were provided to the control insects every 4 days.

Larval molting and mortality were checked daily in the 2nd half of the light regime. The stadia of larvae were determined by checking the shed head capsules daily until the end of the larval stage. Upon reaching the pre-pupal stage (larval body became short and feeding stopped),
the insects were individually transferred to 50-
ml glass vials with around 1.5–2 cm of ver-
miculite at the bottom for pupation. One-
week-old pupae were individually weighed using
a digital weighing scale (FX-300N).

RESULTS
Larval survival
The different diets influenced the survival rate
of the larvae of *H. armigera* (Fig. 1). The final
survival rates (percentage of larvae that survived
until the end of the larval life) decreased in the
following order: highest on okra fruit, Insecta
LF, cotton leaf, tomato leaf, cotton boll,
tomato fruit, corn kernel, and lowest on soy-
bean seed. Larval mortality was highest on toma-
to fruit during the first 4 days of rearing. Also,
high mortality was observed on soybean seed in
the later part of the larval stage. However, in
these stages, fungal growth was observed, which
might have contributed to the high mortalities
of the larvae. On okra fruit during the first 5
days, larval survival was very high, followed by
a sharp reduction by the 10th day, but essen-
tially no decline thereafter. Thus, the final sur-

vival rate on okra was the highest among the
groups tested. Larval survival rate was also high
on cotton leaf by the first 9 days, but declined
sharply thereafter, giving a final survival rate
that was lower than those for insects reared on
okra fruit and Insecta LF. Larvae on Insecta LF
died gradually during the first two weeks of the
larval stage, and 62% of the larvae survived to
pupae. Survival rate in those reared on tomato
leaf, cotton boll, tomato fruit, corn kernel and
soybean seed declined more prominently during
the early larval stage than in those reared on
Insecta LF, resulting in a final larval survival
rate that was lower than 60%.

Larval duration and pupal weight
The duration of the larval period and the
mean pupal weight were significantly affected by
the different larval diets (*p < 0.05*, Scheffe’s *F-
test*) (Table 1). The mean larval duration on
cotton leaf was longest, and significantly longer
than those of the other groups. Those reared on
tomato leaf had a shorter larval period by ca. 4
days than those reared on cotton leaf, but still a
significantly longer larval period than those
reared on the other diets. On the other hand, the
shortest mean larval period between 11 and 13
days was obtained when the larvae were reared
on cotton boll, corn kernel and Insecta LF.

The mean pupal weight was significantly
greater in the individuals reared on Insecta LF
than in those reared on the other diets except
soybean seed. The mean pupal weight of the
individuals reared on tomato leaf was the
lowest, followed by those reared on cotton leaf.

Number of larval stadia
There was a distinct variation in the number
and frequency of the larval stadia on the differ-
ent larval diets (Fig. 2). The number of larval
stadia ranged from 5 to 6 for the individuals fed
on cotton boll, soybean seed, corn kernel and
Insecta LF. Those fed on okra fruit and tomato
fruit had 5 to 7 stadia while those reared on
cotton leaf had 6 to 7 stadia. Though the num-
ber of stadia was the same in the larvae reared
on certain diets, the frequency of larvae which
underwent a particular stadium type varied.
More than 80% of the larvae fed on cotton boll,
corn kernel and Insecta LF had 5 stadia while
only 21% of the individuals fed on soybean
seed had 5 stadia. Among those which exhibited
the 5- to 7-stadia type, about 80% of those
reared on okra fruit had 5 stadia, but only 30%
of the larvae fed on tomato fruit had 5 stadia
and around 66% had 6 stadia. The frequencies
of larval stadium type for the individuals reared
on cotton leaf and tomato leaf were quite similar; most larvae had 6 stadia and one tenth or less had 7 stadia.

**DISCUSSION**

The present study showed that a substantial proportion of the larvae of *H. armigera* survived on all the diets tested, though the survival rates varied greatly (Fig. 1), reflecting their highly polyphagous nature. Okra fruit appeared to be the best host as the survival rates in the early and late larval stage were the highest or second highest among the larvae tested including those fed on the artificial diet Insecta LF. Only the larvae which were reared on okra fruit and Insecta LF had survival rates higher than 60% by pupation. On most food plants tested, larval survival was low in the early stage (1st- to 2nd-stadia). On soybean seed, high mortality was also observed in the larvae approaching the pre-pupal stage. Although larval size was not measured, we observed that the larvae which died in the late larval stage were those which had smaller body size and longer larval period. In spite of changing the diet and the rearing vials daily, fungal growth was noted on the soybean diet, which appeared to have partly contributed to the high mortality of these larvae. Among the groups, the Okinawa population was only used for rearing on soybean and
this may also have influenced our results.

Larval survival in Helicoverpa/Heliothis spp. has been observed to be greatly affected by larval diet. Dhandapani and Balasubramanian (1980) have found that the percentage of *H. armigera* larvae which survived to pupation was greatest on cotton (84%) and lowest on sorghum (70%) at a temperature of 26±2°C, although they failed to specify what parts of the plants were given to the larvae. Sison and Shanower (1994) have demonstrated that *H. armigera* larvae that fed on pigeonpea pods and flowers had a higher survival rate than larvae that fed on leaves of the same plant at a temperature of 22.5±1.5°C. At three temperature levels (20, 25 and 30°C), Nadgauda and Pitre (1983) have found that percentage survival of the 1st-stadium larvae of *H. virescens* was greatest on artificial diet, followed by soybean (trifoliate leaves, blooms and pods), and lowest on cotton (terminal leaves, squares and bolls).

The larval period varied significantly with the diets tested, particularly in the larvae fed on the crops' fruits or seeds, being substantially shorter. This caused heavier pupal weight compared to those reared on the crops' leaves (Table 1). The nature and quality of the larval hosts have been observed to influence the length of the larval period in Helicoverpa/Heliothis spp. Larvae of *H. armigera* fed on corn silks, one-week-old corn cobs (grains on the cob), one-week-old cotton bolls, young dwarf beans, cotton flower buds, and sunflower corolla and receptacles had mean larval periods of 21.8, 22.2, 23.4, 24.5, 25.9 and 33.6 days, respectively (Coaker, 1959). Larvae fed on cotton squares had a mean larval period of 18.4 days (Reed, 1965). Among eight larval diets tested, Dhandapani and Balasubramanian (1980) recorded the shortest and longest larval periods of 17 and 20 days on redgram and tomato, respectively, at a temperature of 26±2°C. However, Singh and Singh (1975) recorded a range of 8 to 12 days or a mean of 10.8 days on tomato green fruit at an average temperature of between 23.34 and 37.47°C. Further, larval period was greatly influenced by the part of the plant fed on. On short-duration pigeonpea, Sison and Shanower (1994) recorded the longest larval period on leaves, followed by flowers and shortest on pods.

In the present study, larval duration on either tomato fruit or leaf was shorter compared to the values of Dhandapani and Balasubramanian (1980) but longer than those of Singh and Singh (1975). Our values on cotton boll and corn kernel were also shorter than the previous results.

Body weight has been observed to differ significantly with diet: the heaviest pupal weights were recorded for larvae fed on cotton and the lightest for those fed on sorghum and tomato (Dhandapani and Balasubramanian, 1980). Larvae given pigeonpea pods resulted in pupae that were 2.6 and 1.5 times heavier than those given leaves and flowers of the same plant (Sison and Shanower, 1994). In *Heliothis zea*, Isley (1935) found that pupal size varied with the nutritive value of the food. The pupae formed by larvae fed on corn kernels weighed about 2.5 times as much as pupae formed by larvae fed on alfalfa foliage. Hardwick (1965) pointed out that the larvae show a decided preference for feeding on the flowers and fruits of their food plants, as indicated by the females ovipositing during the flowering period of the plants. He further mentioned that one of the primary factors governing the feeding habits of the Helicoverpa/Heliothis spp. appears to be the necessity of obtaining food with sufficiently high protein content. This may explain why the larvae who fed on the plants' fruits or seeds became substantially heavier pupae in the present study.

The number of larval stadia in the *Heliothis* spp. varies from 5 to 7 with 6 being most common (Hardwick, 1965) and the nutritive value of the larval diet is one factor which influences this variation (Hardwick, 1965; Nadgauda and Pitre, 1983). In the present study, we recorded 5 to 7 stadia and the frequency of occurrence varied considerably with the larval diets. It appeared that the frequency of the stadium types skewed toward the 5 stadium on the diets which had produced larvae with short durations (cotton boll, corn kernel, Insecta LF and okra fruit). More than 80% of the larvae had 5 stadia on these diets. A large number of the individuals with intermediate larval periods (soybean seed and tomato fruit) and those with long larval durations (cotton leaf and tomato leaf) had
6 stadia. While the 5-stadia type larvae were observed on soybean seed and tomato fruit, the same type of larva was not noted on cotton and tomato leaves. The 7 stadia type was only recorded on okra fruit, tomato fruit, cotton leaf and tomato leaf, with a higher frequency on the last two diets.

As it is known that molting in insects facilitates growth, its frequency may depend on the nutritive value of the diets and may possibly be a mechanism for growth and survival in *H. armigera* which deals with a diverse array of host plants.

The present study showed that *H. armigera* larvae could thrive on poor and good host plants. Fitt (1989) has enumerated three important contributions of polyphagy to the population dynamics and pest status of *Heliothis* (*Helicoverpa*) spp.: (1) populations may develop simultaneously on a number of hosts within a region, (2) populations may develop continuously during suitable periods by exploiting a succession of different cultivated and uncultivated hosts through the season, and (3) populations can persist at low density in seemingly unsuitable areas, since females have a high probability of locating a host able to sustain larval development.

An important subject to clarify in the future is the influence of larval diet on other aspects that contribute crucially to the pest status of *H. armigera*, like migratory propensity and capability.

**ACKNOWLEDGEMENTS**

We sincerely thank Dr. Hisaaki Tsumuki, Dr. Tamotsu Murai, and Mr. Mohammed Hanif Qureshi of the Research Institute for Bioresources, Okayama University, for providing the initial populations of the insects used in this study. The first author wishes to express his deep gratitude to the Ministry of Education, Science, Sports and Culture of Japan (Monbusho) for a scholarship grant.

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