FEEDING ON NON-HOST PLANTS BY THE TOBACCO HORNWORM (*MANDUCA SEXTA* (LEPIDOPTERA: SPHINGIDAE))

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**ABSTRACT**

Feeding responses of *Manduca sexta* larvae reared on artificial diet, a host plant (jimsonweed) and a non-host plant (cowpea) were tested with leaves of 4 selected non-host plants and compared. Larvae reared on diet and the host plant fed equally on collard, dandelion and cowpea while the diet-reared larvae found mullein more acceptable than did the Jimsonweed-reared larvae. Both groups of larvae found mullein and collard significantly more acceptable than cowpea or dandelion. Larvae reared on the non-host plant readily ate all 4 test plants.

Acceptance of non-host plants increased the longer the larvae fed on cowpea. Larvae were fed initially on cowpea for varying lengths of time and then transferred to either diet or Jimsonweed. Acceptance of the test plants was not as great and took place more slowly in larvae transferred to Jimsonweed. The possible chemosensory basis of this feeding behavior is discussed.

**RESUMEN**

Las reacciones alimentarias de las larvas de *Manduca sexta* criadas con dieta artificial, una planta hospedera (hierba hiedionda o *Datura stramonium*), y una plante que no es hospedera (caupí o *Vigna sinensis*) se examinaron con los de cuatro plantas seleccionadas que no son hospederas, y se compararon. Las larvas criadas con dieta y con la planta hospedera se alimentaron igualmente de la berza común (*Brassica oleracea*), amargón (*Parasaceum officinale*), y caupí, mientras que las larvas criadas con dieta aceptaron mejor el gordolobo (*Verbasum thapsus*) que las larvas criadas con hierba hiedionda. Ambos grupos de larvas aceptaron el gordolobo y la berza común mucho mejor que el caupí o que el amargón. Las larvas criadas con plantas que no son hospederas se alimentaron fácilmente de las cuatro plantas experimentales.

Mientras más tiempo las larvas se alimentaron del caupí, más aumentó la aceptación de plantas que no son hospederas. Inicialmente, las larvas fueron alimentadas con caupí por distintas duraciones de tiempo, y entonces se transferieron a dieta o hierba hiedionda. La aceptación de las plantas experimentales fue menor y más lenta con larvas transferidas a la hierba hiedionda. Se discute la posibilidad de una base químiosensorial que explique este comportamiento.

The influence of feeding experience on host plant selection has been studied in a number of oligophagous insects. Larvae of oligophagous
Lepidoptera species can distinguish between species of host plants and often exhibit a clear preference for the plant on which they have had previous feeding experience (Hanson 1976, Hovanitz and Chang 1965, Stadler and Hanson 1976). The effects on feeding behavior of an "abnormal" food—artificial diet—has also been studied (Stadler and Hanson 1978). Oligophagous insects can sometimes be reared on plants outside their normal range of hostplants when their chemoreceptors are destroyed (Waldbaeur 1964). In this paper we examine the feeding behavior of an oligophagous insect reared without surgical alteration on a non-host plant.

Although the tobacco hornworm, *Manduca sexta* (Johanssen), is normally host specific on solanaceous plants, larvae will feed on other plants under stress of starvation (Yamamoto and Fraenkel 1960, Dethier 1980). They will also feed on certain non-host plants such as mullein or dandelion when the maxillae and the chemoreceptors attached to them are extirpated (Waldbaeur and Fraenkel 1961, Waldbaeur 1962, Schoonhoven and Dethier 1966). However, it is not necessary to starve or maxillectomize larvae in order to get them to feed on non-host plants. For example, when reared on diet the larvae will feed on non-host plants like cabbage and plantain as though they had lost their specificity (Schoonhoven 1967, 1969; Dethier, 1980). This apparent lack of specificity in diet-reared larvae could be explained as an inductive change occurring in the 1st instar (Yamamoto 1974). The evidence indicates that newly hatched larvae are polyphagous and will feed on many kinds of non-host plants. If they are reared on tomato, a host, in the 1st instar, they are induced into the oligophagous state in subsequent instars and they feed specifically on solanaceous plants. If they are reared on diet, on the other hand, they retain to some extent the polyphagous state of the 1st instar. Yamamoto (1974) also observed that larvae fed and developed on cowpea, a non-host plant. The purpose of this study was to compare the feeding specificity of 5th instars with feeding experience on jimsonweed (a host plant) to 5th instars with feeding experience on cowpea and artificial diet. These comparisons can contribute to a better understanding of how early experience influences the feeding behavior of phytophagous insects.

**Material and Methods**

The larvae used in the experiments were obtained from an inbred culture maintained in the laboratory at North Carolina State University for over 6 years. The rearing procedure has been described previously (Yamamoto 1969). The host plant used for rearing was jimsonweed (*Datura stramonium* L., Solanaceae).

Test plants used in the experiments were collard (*Brassica oleracea* L., Cruciferae), dandelion (*Taraxacum officinale* L., Asteraceae), mullein (*Verbascum thapsus* L., Scrophulariaceae), and cowpea (*Vigna sinensis* L., Fabaceae). Other than dandelions, the plants were grown from seeds. Dandelions were collected as mature plants in the field and transplanted into pots. All plants were greenhouse grown.

The acceptability of these test plants to newly hatched larvae was determined by confining the larvae in 9 cm petri dishes with enough leaves to cover the bottom of each dish. A moistened filter paper lining the bottom of the dish kept the leaves succulent. Ten larvae were introduced into each dish and each test was replicated 10 times. After 24 hours, the number of
Flowers & Yamamoto: Feeding by Manduca sexta

larvae feeding on the leaves was counted. Feeding was positive when crops and guts of the translucent larvae showed green leaf matter. Acceptability of dandelion was determined on 3 different occasions since the plants were brought in from the wild.

Larvae in the 5th instar were used to determine the effect of dietary experience on the acceptability of 4 non-host plants. The larvae were confined individually in petri dishes just prior to their molting to the 5th instar. After molting had occurred, 4 plant discs, one each of muillein, collard, cowpea, and dandelion, which were cut from fresh leaves with an 8 mm cork borer, were placed in each dish. The dishes were lined with moistened filter paper. After 24 hours, the area consumed from each disc was visually estimated and recorded on a relative scale of 0 to 1 by increments of 0.25. A disc completely consumed was rated 1 and a disc untouched was rated 0. Although more precise photometric methods for determining leaf areas consumed are available (Kogan and Goeden 1969), estimates were made because in most instances the larvae either ate a leaf disc completely or not at all. A feeding index was calculated for each plant species offered to each experimental group of larvae by dividing the total rating of the plant by the number of larvae in the group. Thus the feeding index represents the mean amount eaten by larvae in an experimental group. For graphic purposes, the feeding index was multiplied by 100. Larvae reared to 6th instar on cowpea, jimsonweed and artificial diet were tested in this way.

After the changes in acceptance of the test plants by cowpea-reared larvae were observed, 3 additional experiments were conducted. In the first, batches of larvae were reared on cowpea for 1 and 2 instars and then switched to diet on which they fed until testing in the fifth instar. In the second experiment, batches of larvae were reared on cowpea for 1, 2 and 3 instars and then switched to jimsonweed and reared until the fifth instar. The object of these experiments was to see what effect increasing length of exposure to cowpea had on feeding behavior.

The third experiment consisted of transferring cowpea-reared larvae to dandelion after each molt and noting the percent that fed. This test was to measure the acceptability of dandelion to larvae exposed only to cowpea.

The data collected for the feeding tests were analyzed statistically by means of a split-plot design. The individual larva was considered as a whole plot and the 4 test plants were considered as sub-plots. Analysis of variance was calculated for each group of larvae. From these, 95% confidence limits were calculated for the feeding indices shown in Fig. 1-3.

Results

Feeding responses of 1st instars. The results of the acceptance tests of 1st instar larvae to the 4 non-host plants are given in Table 1. Collard and mullein were accepted by more than 80% of the larvae and cowpea was accepted by 57%. Dandelion was accepted by 5% of the larvae in one determination and by none in 2 other determinations. The acceptance of 3 out of the 4 non-host plants by more than half the larvae show the polyphagous nature of the newly hatched larvae. Rejection of dandelion may be due to a high concentration of deterrents in this plant. These responses of the 1st instars form the basis for evaluating behavioral changes that are expressed by the 5th instars.
Fig. 1. Feeding indices of 5th instars of the tobacco hornworm reared on diet, jimsonweed and cowpea. Plants tested: P. cowpea; D. dandelion; C. collard; M. mullein. Bottom line gives number of larvae tested in each experiment. Vertical lines at top of bars represent 95% confidence intervals.

Fig. 2. Feeding indices of 5th instars of the tobacco hornworm reared initially on cowpea and then on diet. Numbers following “cowpea” and “diet” represent larval instars feeding on each. Other symbols as in Fig. 1.
Fig. 3. Feeding indices of 5th instars of the tobacco hornworm reared initially on cowpea and then on jimsonweed. Numbers following "cowpea" and "jimsonweed" represent larval instars feeding on each. Other symbols as in Fig. 1.

<table>
<thead>
<tr>
<th>Plant</th>
<th>No. of Larvae</th>
<th>Percent Feeding on Plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cowpea</td>
<td>100</td>
<td>57.0</td>
</tr>
<tr>
<td>Dandelion (greenhouse)</td>
<td>100</td>
<td>5.0</td>
</tr>
<tr>
<td>Dandelion (greenhouse)</td>
<td>90</td>
<td>0</td>
</tr>
<tr>
<td>Dandelion (wild)</td>
<td>90</td>
<td>0</td>
</tr>
<tr>
<td>Collard</td>
<td>100</td>
<td>88.0</td>
</tr>
<tr>
<td>Mullein</td>
<td>100</td>
<td>83.0</td>
</tr>
</tbody>
</table>

Feeding responses of 5th instars. The feeding indices (Fig. 1) of jimsonweed-reared larvae and diet-reared larvae were significantly different only with respect to mullein. Jimsonweed-reared larvae consumed less mullein than did diet-reared larvae. The indices for cowpea and dandelion for both groups of larvae, however, were low in comparison to the indices for mullein and collard. It was expected that, because of induced oligophagy, jimsonweed-reared larvae would feed less on mullein and collard than would diet-reared larvae but this expectation was not completely borne out. There was no difference in the consumption of collard. The feeding indices for dandelion and cowpea of cowpea-reared larvae were significantly different from those of diet-reared and jimsonweed-reared larvae. It was expected that cowpea-reared larvae would consume mullein, collard, and cowpea but the high consumption of dandelion was not expected.

The feeding indices of larvae reared on cowpea before being transferred
to diet are shown in Fig. 2. Larvae reared exclusively on diet served as controls. Larvae which had fed on cowpea for 1 or 2 instars consumed significantly more mullein, cowpea and dandelion than did the controls. The feeding indices of larvae transferred to jimsonweed after feeding for 1, 2 or 3 instars in cowpea are shown in Fig. 3. Control larvae were fed exclusively on jimsonweed. There was not significant difference in consumption of all 4 test plants between control larvae and larvae reared on cowpea for the 1st instar and on jimsonweed for the 2nd to 4th instars. However, significantly more mullein, dandelion, and cowpea were consumed by larvae reared on cowpea for 2 or 3 instars before being transferred to jimsonweed.

Feeding responses of cowpea-reared larvae when transferred to dandelion. The acceptance of dandelion by nearly half the larvae occurred in the 4th instar and by more than half in the 5th instar (Table 2). Third instars scarcely accepted dandelion.

**DISCUSSION**

Oligophagy or hostplant specificity in tobacco hornworm larvae is not strictly inherited and results in part from their feeding on a hostplant (Yamamoto 1974). The change to oligophagy occurs in the 1st instar and the measurable effect of the change is the preference of older larvae for the species of host plant on which they have been feeding (Hanson 1976, Stadler and Hanson 1976). Prior to the change, the need for stimulating compounds is not essential for feeding and newly hatched larvae will feed on many kinds of non-host plants. Table 1 records their feeding on mullein, cowpea and collard; but not on dandelion, which may possess deterrents in high concentrations. When larvae are reared on diet, they are not induced into oligophagy on Solanaceae and therefore their acceptance of non-host plants is expected to be similar to the 1st instars. (However, Stadler and Hanson (1978) have shown that the hornworm diet that we used is not 'neutral' and contains chemicals which induce subsequent feeding preferences). On the other hand, larvae reared on host plants should reject or at most feed feebly on non-host plants like mullein, collard, or cowpea because these plants presumably lack specific token stimulants. The expected difference between diet-reared and jimsonweed-reared larvae was not borne out completely (Fig. 1). Both groups ate similar amounts of collard. Jimsonweed-reared larvae ate less mullein, as expected, than did diet-reared larvae, but they also ate more dandelion and cowpea. The degree of induced oligophagy, that is to say the degree to which non-host plants are rejected by 5th instars, depends on the species of host plant on which they are reared (Yamamoto, 1974). For

**TABLE 2. ACCEPTANCE OF DANDELION BY COWPEA-REARED TOBACCO HORNWORM LARVAE.**

<table>
<thead>
<tr>
<th>Instar</th>
<th>No. of Larvae</th>
<th>Percent Feeding on Dandelion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st (control)</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>2nd</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>3rd</td>
<td>25</td>
<td>4.0</td>
</tr>
<tr>
<td>4th</td>
<td>29</td>
<td>47.5</td>
</tr>
<tr>
<td>5th</td>
<td>16</td>
<td>62.4</td>
</tr>
</tbody>
</table>
example, tomato and potato are good inducing plants whereas tobacco and jimsonweed are weak inducing plants. If larvae had been reared on tomato instead of jimsonweed their rejection of mullein and collard would probably have been more obvious than was the case with jimsonweed-reared larvae. Stadler and Hanson (1976) found that tomato contains water soluble feeding deterrents that are lacking in Jerusalem cherry (Solanum pseudocapsicum), another host plant of the hornworm. In tomato, at least, effective induction appears to be related to the presence of deterrents as well as stimulants. It is of interest that both jimsonweed-reared and diet-reared larvae found cowpea highly unpalatable. Hence, not only jimsonweed-reared but diet-reared larvae were subjected to modification of their feeding behavior.

The test plants, particularly dandelion, became more acceptable to hornworm larvae that had fed for at least one instar on cowpea. In fact, 2 instars of feeding on cowpea were necessary to overcome the effects of later feeding on jimsonweed (Fig. 2 and 3). The observed changes could be due to the desensitization of deterrent chemoreceptors which are exposed to presumed deterrents in cowpea. The desensitized chemoreceptors would thus be insensitive to deterrents in dandelion, assuming that deterrents in both plants affect the same chemoreceptors. Desensitization of chemoreceptors in caterpillars has been shown to occur when larvae are reared on diets containing feeding stimulants or deterrents (Ishikawa et. al. 1969, Schoonhoven 1969). For example, when a tobacco hornworm larva was fed diet containing inositol, a feeding stimulant, the inositol chemoreceptor fired at a lower rate than did the inositol chemoreceptor of a control larva. When salicin, a glycoside with deterrent activity, was fed to a larva, less firing was recorded in the deterrent chemoreceptor when compared to the firing in the deterrent chemoreceptor of a control larva (Schoonhoven 1969). It was found too, that larvae reared on tomato and potato were less sensitive to salicin than larvae reared on diet (Schoonhoven 1969), indicating that host plants may contain chemicals which desensitize the deterrent chemoreceptors. This may explain the results shown in Fig. 1. where jimsonweed-reared larvae ate more of dandelion and cowpea than did diet-reared larvae, although for practical purposes, these plants are rated unacceptable to both types of larvae. They are unacceptable to diet-reared larvae because the deterrents are high but their unacceptability to jimsonweed-reared larvae does not appear to be the same. We have observed that dandelion, for example, is readily eaten by jimsonweed-reared larvae if it is soaked in an extract of tomato but it is still rejected by diet-reared larvae.

High acceptance of dandelion by larvae fed on cowpea in Fig. 2 and 3 cannot be entirely due to the effects of cowpea alone. Table 2 shows that cowpea-reared larvae do not accept dandelion to any great extent until after the third instar. Yet larvae reared for only 2 instars on cowpea then fed on diet of jimsonweed show greater acceptance of dandelion than Table 2 would indicate. Again, the desensitizing of deterrent receptors by constituents not only of cowpea but also of diet and jimsonweed could be the explanation.

This study indicates that the naive (uninduced) 1st instar tobacco hornworm has the potential for either oligophagy or polyphagy. If it feeds on a solanaceous plant, it becomes oligophagous because of inductive processes which lead to its requirement for stimulants and its habituation to deter-
rants. If it feeds on a non-solanaceous plant on which it can develop it can extend its range of food plants because it seems even less sensitive to deterrents. In nature, it appears that adults oviposit primarily on Solanaceae and thus hatching larvae become induced to feed on this family of plants. It would be interesting to investigate whether the acceptance of non-host plants demonstrated by our laboratory animals occurs or has any practical survival value in wild populations.

ACKNOWLEDGEMENTS

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