INDUCTION OF HOSTPLANT SPECIFICITY IN THE TOBACCO HORNWORM, \textit{MANDUCA SEXTA}

R. T. YAMAMOTO

Department of Entomology, North Carolina State University, Raleigh, North Carolina 27607, U.S.A.

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Abstract—The hostplant specificity of the tobacco hornworm, \textit{Manduca sexta}, is not inherited but is induced. Newly hatched larvae are polyphagous and will feed on many kinds of non-hostplants although they are not able to grow on them. They become oligophagous when they are reared on solanaceous plants but remain polyphagous when reared on diet. The critical period for induction is in the first instar. The induced oligophagous behaviour can be reversed by forcing larvae to feed on diet.

INTRODUCTION

The utilization of token stimulants by oligophagous insects for discriminating hostplants from non-hostplants is well established (Fraenkel, 1959, 1969; Dethier, 1970). Token stimulants, nevertheless, are not essential for feeding since many oligophagous insects can be reared on artificial diets lacking hostplant tissues or extracts. However, insects reared on diets may show behavioural differences from those reared on plants. Thus Schoonhoven (1967a) reported that larvae of the tobacco hornworm, \textit{Manduca sexta}, when reared on diet, lost their specificity to solanaceous plants and fed readily on cabbage and plantain. Previously, the ready acceptance of non-hostplants by the larvae could only be accomplished by removing the maxillae on which the primary gustatory chemoreceptors were situated (Waldbauer and Fraenkel, 1961; Schoonhoven and Dethier, 1966). This change of behaviour from oligophagy to modest polyphagy in diet-reared larvae was attributed to alterations in the sensitivity of maxillary chemoreceptors to chemicals in plants (Schoonhoven, 1969a). I have recently reinvestigated this apparent loss of feeding specificity in the tobacco hornworm by measuring the feeding responses of each instar to non-hostplants. The findings indicate that larvae in the first instar are polyphagous and are able to feed on many non-hostplants. They become oligophagous when they feed on a hostplant in the first instar, hence older hostplant-reared larvae show oligophagous behaviour whereas diet-reared larvae, not being induced to oligophagy, continue to feed on non-hostplants like the first instars. The evidence for this inductive change in the larvae is presented here.

MATERIALS AND METHODS

The larvae used in the experiments were obtained from a culture maintained continuously in this laboratory for the past 6 years (Yamamoto, 1969). The plants
were grown and maintained under nearly identical growing conditions in a greenhouse. Only young succulent parts of the plants were used in the feeding tests.

In the acceptance–rejection tests, mullein (*Verbascum thapsus* L.) (family Scrophulaceae) was used because it was highly acceptable to newly hatched larvae. Mullein collected in the wild was less acceptable and at times unacceptable. Where applicable, $\chi^2$ tests of significance were conducted on feeding responses of experimental groups of larvae with respect to performances by control groups of larvae, which were run concurrently in each series of tests.

The feeding experiments were conducted in a walk-in cabinet with a constant temperature of 26°C, r.h. of 60%, and a photoperiod of 16 hr light and 8 hr dark.

**EXPERIMENTAL RESULTS**

*Feeding responses of newly hatched larvae*

In an earlier work, the feeding specificity of the tobacco hornworm to solanaceous plants was established by using large larvae reared on hostplants, principally tomato, in feeding tests. The larvae fed readily and interchangeably on most solanaceous plants tested and fed feebly or rejected non-solanaceous plants (YAMAMOTO and Fraenkel, 1960a). Newly hatched larvae were now tested on 14 non-solanaceous plants and 1 solanaceous plant. The plants were placed in 10 cm diameter Petri dishes which were lined with moistened filter paper. To each dish, 10 larvae were added. Replicate dishes were conducted for all plants. The larvae were examined for evidence of feeding under a dissecting microscope every 2 hr for the first 8 hr and at 4-hr intervals thereafter up to 24 hr. The evidence for feeding was the presence of plant parts in the crop or gut of the translucent larvae. Attempts were also made to rear the larvae on the non-hostplants.

Eight of the 14 species of non-solanaceous plants were eaten by more than 50 per cent of the test larvae within the 24-hr period of observation (Table 1). The larvae did not feed on cotton, burdock, peanut, oxalis, and sweet potato. Except on mullein and cow pea, larvae did not grow beyond the second instar on the non-solanaceous plants. Mullein supported larvae to the fourth instar and cow pea supported larvae until growth was completed.

Mullein was accepted nearly as readily as the control plant, tomato, since 50 per cent or more of the larvae were feeding on it 2 hr after the experiment was initiated. Collard, mulberry, and plantain were acceptable within 6 hr. Alfalfa, cow pea, and snapbean required 24 hr of exposure before being accepted by 50 per cent or more of the larvae. Strawberry and dandelion were accepted by only 15.6 and 5.0 per cent of the larvae respectively. These feeding tests show the non-specific feeding responses of newly hatched larvae.

*Choice of plants by larvae in four-choice situations*

The objective of this experiment was to find out what plants newly hatched larvae and older larvae would choose when confronted with a choice of several plants. The older larvae were reared on tomato, cow pea, and diet and they were used soon after moulting into the second, third, and fourth instars. Fifth instars
**TABLE 1—Feeding responses of newly hatched tobacco hornworm larvae on a hostplant, on non-hostplants, and on diet**

<table>
<thead>
<tr>
<th>Plant</th>
<th>Number of larvae</th>
<th>Per cent feeding (24 hr)</th>
<th>Time 50% of larvae feeding (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomato <em>Lycopersicum esculentum</em> Mill. (family Solanaceae)</td>
<td>96</td>
<td>100-0</td>
<td>2</td>
</tr>
<tr>
<td>Mullein <em>Verbascum thapsus</em> L. (family Scrophulaceae)</td>
<td>110</td>
<td>93-6</td>
<td>2</td>
</tr>
<tr>
<td>Collard <em>Brassica oleracea</em> L. (family Cruciferae)</td>
<td>100</td>
<td>93-0</td>
<td>4</td>
</tr>
<tr>
<td>Mulberry <em>Morus rubra</em> L. (family Moraceae)</td>
<td>50</td>
<td>80-0</td>
<td>6</td>
</tr>
<tr>
<td>Plantain <em>Plantago major</em> L. (family Plantaginaceae)</td>
<td>113</td>
<td>90-5</td>
<td>6</td>
</tr>
<tr>
<td>Alfalfa <em>Medicago sativa</em> L. (family Leguminosae)</td>
<td>95</td>
<td>51-0</td>
<td>24</td>
</tr>
<tr>
<td>Cow pea <em>Vigna sinensis</em> (L.) (family Leguminosae)</td>
<td>102</td>
<td>72-5</td>
<td>24</td>
</tr>
<tr>
<td>Snapbean <em>Phaseolus vulgaris</em> L. (family Leguminosae)</td>
<td>100</td>
<td>69-0</td>
<td>24</td>
</tr>
<tr>
<td>Strawberry <em>Fragaria virginiana</em> Duch. (family Rosaceae)</td>
<td>32</td>
<td>15-6</td>
<td>—</td>
</tr>
<tr>
<td>Dandelion <em>Taraxacum officinale</em> Weber (family Compositae)</td>
<td>120</td>
<td>5-0</td>
<td>—</td>
</tr>
<tr>
<td>Diet</td>
<td>101</td>
<td>90-0</td>
<td>14</td>
</tr>
</tbody>
</table>

were not used because sufficient plants for rearing them were not available. The arena was a 10 cm diameter Petri dish, lined with moistened filter paper, and containing four 12 mm discs of plants. The discs were cut out from the leaves of tomato, mullein, collard, and cow pea with a corkborer. The discs were arranged equidistant from each other and the larvae were placed in the centres of the dishes. Five newly hatched larvae were used in each dish. Larvae in the second, third, and fourth instars were used individually. A larva was recorded as having made a choice of a plant when it was found feeding on the same species of plant during two consecutive observations, 1 hr apart. A minimum of 50 larvae in each instar and each dietary experience was used. The results are represented graphically in Fig. 1.

In each series of determination, as shown in Fig. 1, the pattern of plants chosen by the first instars was consistent. The greater number of larvae chose tomato (60 to 75 per cent), a lesser number chose mullein (17 to 32 per cent), and the remainder chose collard (2-5 to 8-0 per cent) and cow pea (0 to 5-0 per cent). The pattern of plants chosen by older larvae, however, varied with the dietary experience, particularly in respect to feeding on the non-hostplants. Larvae reared on tomato chose tomato to the near exclusion of the non-hostplants in the second and third instars, and chose tomato exclusively in the fourth instar. Larvae reared on cowpea (second to fourth instars) chose tomato (51 to 70 per cent), then collard and cow pea (28-5 to 39-5 per cent), and finally mullein (2-0 to 7-0 per cent). Larvae reared on diet (second to fourth instars) also chose tomato principally (55-0 to 63-0 per cent), then mullein and collard (35-0 to 44-5 per cent) and shunned cow pea in all three instars.
Several interesting points were revealed in the choice tests. Firstly, newly hatched larvae did not invariably choose tomato; secondly, larvae reared on cow pea and diet continued to choose and feed on non-host plants; and, thirdly, larvae reared on tomato chose tomato nearly exclusively.

**Inductive changes in feeding behaviour**

The feeding behaviour of larvae reared on tomato was examined further by subjecting them to a series of three acceptance–rejection tests on mullein, a plant which was highly acceptable to newly hatched larvae (Table 1). In the first experiment, the larvae were placed on tomato in the first instar and tested subsequently when they grew and ecdysed into second, third, fourth, and fifth instars. Responses of larvae reared on diet were also run concurrently. In these tests, except for the first instars, each larva was confined in a 10 cm diameter Petri dish, which was lined with a moistened filter paper and which contained one 12 mm disc of mullein. After 24 hr of exposure, the acceptance or rejection of mullein was recorded. There was no difference in acceptance when the larvae were exposed to mullein for 48 hr. If mullein was acceptable to a larva, it was completely consumed or a larva was found feeding on the disc when examined. If mullein was unacceptable to a larva, it was unmarked or had a ragged edge caused by bites. A minimum of 70 larvae in each instar and each dietary experience was used. Newly hatched
larvae were tested by placing 10 of them per Petri dish containing mullein and recording their responses 24 hr later. The performance of these larvae served as controls for the responses of the older larvae.

In this experiment all or 100 per cent of the newly hatched larvae accepted mullein. They are represented in Fig. 2 as two parallel and equal columns and the per cent acceptances of mullein by older larvae are also shown in the figure. It is clear from the figure that the percentages of tomato-reared larvae in all subsequent instars accepting mullein are significantly lower than the first instars whereas the percentages of diet-reared larvae, except in the second instar, are not. Thus feeding on tomato induces a change leading to rejection of mullein.

![Bar graph showing acceptance of mullein by Manduca larvae reared on tomato and diet](image)

**Fig. 2.** Acceptance of mullein by *Manduca* larvae reared on tomato and on diet. Seventy larvae were used per determination in the second, third, and fourth instars.

The objective of the second experiment in this series was to determine the critical period for inducing rejection of mullein. Larvae were placed on diet after hatching and were transferred to tomato soon after ecdysis into the second, third, and fourth instars respectively. Larvae transferred in the second instar fed for three instars, those in the third for two instars and those in the fourth for one instar on tomato before being tested on mullein shortly after ecdysis into the fifth instar. The larvae, as before, were confined individually with a mullein plug in Petri dishes. Larvae reared exclusively on tomato from the first instar served as controls. Forty larvae were used for each determination. The results (Fig. 3) show that larvae reared on diet for at least the first instar before being transferred to tomato did not reject mullein as significantly as did the control larvae which were reared on tomato exclusively. The first instar appears to be sensitive to inductive changes in feeding behaviour.
FIG. 3. Acceptance of mullein by fifth instar *Manduca* larvae reared initially on tomato and transferred subsequently to diet. Forty larvae were used in each determination.

The reversibility of the induced change was tested by rearing larvae initially on tomato then transferring them to diet in the second, third, and fourth instars respectively. They were tested for acceptance of mullein soon after moulting into the fifth instar. The method of evaluating acceptance of mullein was the same as before. The control group of experiment 3 (Fig. 3) served as control for this experiment also since both experiments were run concurrently. The results (Fig. 4)

FIG. 4. Acceptance of mullein by fifth instar larvae reared initially on diet and transferred subsequently to tomato. Forty larvae were used in each determination.
showed that significant percentages of larvae transferred to diet from tomato accepted mullein when compared to the controls. Thus the induced change in feeding behaviour can be reversed by forcing larvae to feed on diet.

**Responses of larvae to extracts of tomato**

It was assumed that larvae in the induced state were responding to token stimulants since they fed interchangeably on solanaceous plants. This assumption was tested by giving tomato-reared and diet-reared larvae a choice of artificial diets with and without a purified extract of tomato, containing token stimulants (Yamamoto and Fraenkel, 1960b). The test diets were 9 mm round and 3 mm thick. One third instar larva was used per Petri dish containing one test and one control diet. Newly hatched larvae, 10 to a dish, served as controls. After 24 hr, the choice of diets made by the larvae was recorded. This was easily determined because the larvae did not consume the diets completely and were usually found feeding on them. The results (Table 2) show that significantly more tomato-reared larvae (83.5 per cent) chose diet with an extract of tomato than did diet-reared larvae (52.5 per cent) or newly hatched larvae (54.6 per cent). Thus it would seem that induced larvae became responsive to token stimulants in tomato.

<table>
<thead>
<tr>
<th>Instar</th>
<th>No.</th>
<th>Diet</th>
<th>Diet + tomato extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>First (control)</td>
<td>97</td>
<td>45.4</td>
<td>54.6 **</td>
</tr>
<tr>
<td>Third (diet-reared)</td>
<td>38</td>
<td>47.5</td>
<td>52.5 **</td>
</tr>
<tr>
<td>Third (tomato-reared)</td>
<td>36</td>
<td>16.5</td>
<td>83.5 **</td>
</tr>
</tbody>
</table>

** Significant different from control at 1 per cent level.

**DISCUSSION**

The apparent loss of feeding specificity by diet-reared larvae (Schoonhoven, 1967a) assumed or implied that this trait is inherited in the tobacco hornworm. This assumption is not entirely correct for the larvae, upon hatching, showed polyphagous rather than oligophagous traits. They fed on many kinds of non-hostplants (Table 1) and in four-choice situations, sufficient percentages of larvae, varying from 22 to 39 per cent in three separate trials (Fig. 1), chose non-solanaceous plants even in the presence of tomato, a natural hostplant. They likely chose and fed on the non-hostplants because they were not attuned to a particular chemical, a token stimulant. Rather, their feeding responses might have been governed by summed inputs of various inhibitory and stimulatory chemicals (Schoonhoven, 1969b). Tomato, chosen by a large percentage of the larvae (Fig. 1), is apparently highly palatable because it contains more stimulatory than inhibitory chemicals. Cow pea, on the other hand, although acceptable under forced conditions, was least
chosen under choice situations because inhibitory chemicals nearly equaled stimulatory chemicals. When less palatable solanaceous plants such as tobacco and *Nicandra* were used in place of tomato in choice situations, the percentages of larvae choosing tobacco and *Nicandra* was only 54 and 33 per cent respectively. The larvae choosing non-hostplants correspondingly increased. The percentages were obtained in single trials. *Nicandra* possesses a feeding deterrent, the concentration of which is not high enough in fresh plants to cause total rejection by the larvae (NALBANDOV et al., 1964). It would seem, on the basis of the choice experiments and forced feeding of the young larvae on non-hostplants, that they are very sensitive to deterrent chemicals and thus when confronted with a choice of plants, they fed on the least deterrent ones. Mullein and tomato apparently contain little or no deterrents.

Older larvae reared on diet or cow pea retained the polyphagous behaviour of the newly hatched larvae but larvae reared on tomato sought out tomato (Fig. 1) or rejected a palatable plant (Fig. 2). They also chose diets containing purified extracts of tomato over ordinary diets whereas newly hatched larvae or diet-reared larvae did not (Table 2). They will also feed on mullein soaked in the extract, a response which has now been refined into a bioassay. These oligophagous traits indicate that their feeding behaviour is now regulated by a specific chemical in tomato or other solanaceous plants, a token stimulant. The token stimulant is the key factor in their assessment of plants under normal conditions of feeding. In nature, the oligophagous behaviour of the larvae is assured because the adults do not oviposit randomly but mainly on solanaceous plants, oviposition being elicited by a specific chemical in the hostplants. The newly hatched larvae therefore feed on solanaceous plants and acquire the oligophagous behaviour after completing growth through the first instar. Their induced behaviour keeps the insect on plants which provide them with the best possible chance for growth. As shown in Table 1, newly hatched larvae may feed on a wide spectrum of plants but, with the exception of tomato and cow pea, they cannot complete growth on them. Also I have found that diet-reared larvae transferred to cabbage and plantain in the fourth larval instar were not able to complete growth on these plants. Thus oligophagy appears to be a survival mechanism for the tobacco hornworm.

Some past work by others provides tentative possible mechanisms for the inductive process leading to oligophagy. SCHOONHOVEN (1969a, b) located a chemoreceptor in the tobacco hornworm, the ‘s’ cell, which might be responsive to token stimulants in tomato. The ‘s’ cell is one of four cells in the medial sensillum styloconicum of the maxilla, and its sensitivity to a select group of chemicals, if these are isolated, may correspond to the specificity and sensitivity of two chemoreceptors for mustard oil glycosides in the cabbage butterfly larva (SCHOONHOVEN, 1967b). DETHIER (1970) has further suggested that chemoreceptors responsive to token stimulants may have configurational sites which can accommodate one, several related, or many different kinds of compounds. The behaviour corresponding to the configurational sites of the receptors would be monophagy (one), oligophagy (several related), and polyphagy (many different). If a chemoreceptor
with a particular site is inherited rather than the behaviour, then the inductive process could possibly involve (1) activation or alteration of the receptor site, (2) perceptual coding at higher integrative centres (HAILMAN, 1970), or (3) combination of both. SCHONHOVEN (1969a) showed that chemoreceptor sensitivity to chemicals can change, usually becoming less sensitive, when the chemicals are present in the food of the larvae. Thus it is possible for the ‘s’ cell to become altered when it comes in contact with token stimulants. However, the critical period for inducing oligophagy is in the first instar (Fig. 3) and it seems, therefore, that perceptual coding at higher integrative centres, possibly in the suboesophageal ganglion or in the brain, is the more likely inductive process. This process is reversible (Fig. 4), although it is not likely to occur in nature since the larvae would reject non-hostplants.

Insects may also express preference for a particular species of plant from among a group of acceptable hostplants. Preference of this type is also induced by prior feeding on a plant (TAKATA, 1959; HOVANITZ and CHANG, 1963; JERMY et al., 1968). In the tobacco hornworm, for example, Jermy and co-workers showed that larvae reared on tomato and tested subsequently in the fifth instar preferred tomato over other acceptable solanaceous plants when given a choice. In this instance, the non-preferred plants were not rejected and feeding also occurred on them. Induced preference differs from induced oligophagy because in the latter case plants acceptable at an early instar become unacceptable to induced larvae. Another difference is that induced preference was not reversed by feeding larvae on diet whereas induced oligophagy was (Fig. 4). It is obvious that both inductive processes occur in the same individual when it feeds on a particular species of hostplant.

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