

The effects of larval experience with a complex plant latex on subsequent feeding and oviposition by the cabbage looper moth: *Trichoplusia ni* (Lepidoptera: Noctuidae)

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Summary. Leaf disc choice and oviposition bioassays were used to examine the effects of larval experience with a *Hoodia gordonii* latex on subsequent behaviors. The latex deterred feeding and oviposition by “naïve” cabbage looper (*Trichoplusia ni*, Noctuidae) larvae and moths with no previous exposure to the material. “Experienced” insects, reared on a diet with the *H. gordonii* latex (1000 ppm), exhibited lesser feeding deterrence relative to naïve insects. Experienced female moths actually preferred to lay eggs on treated rather than control leaves. There was no observed transfer of behavioral preferences from experienced parents to their offspring. Our results suggest that moths may be acquiring oviposition preferences from larval feeding experience as described by Hopkins’ host selection principal (HHSP) or through chemical legacy.

Key words. Antifeedant – feeding deterrence – oviposition deterrence – *Trichoplusia ni* – *Hoodia gordonii*

Introduction

The information afforded by plant chemistry is critical in guiding the process of host selection by phytophagous insects. The decision to accept or reject a plant as a suitable host is not always based on a few key stimuli, but often on a large variety of stimulatory and inhibitory plant chemicals acting together (Schoni *et al.* 1987). The chemistry of a plant can be highly variable, as can insect responses to plant constituents as a result of growth characteristics, genetic variation, and/or environmental factors (Renwick, 2001). Such variability in nature may provide windows of opportunity for non-adapted insects to utilize a new plant, or for a plant to become resistant to a normally adapted herbivore.

For lepidopterans, host finding and acceptance is largely the responsibility of the female adult. She is usually responsible for selecting an oviposition site that is optimal for the newly hatched larvae to maximize juvenile survival on that host (Renwick, 2001). Females live for a few weeks at best; consequently, they possess behavioral and physiological mechanisms to help them find suitable oviposition sites. The choice of oviposition site is directed by visual and chemical

cues, and acceptance may be governed either by stimulating signals or by the absence of deterrents (Anderson *et al.* 1995; Renwick & Radke, 1988; Papaj and Prokopy, 1986; Rausher, 1978; Feeny *et al.* 1983, Renwick and Radke, 1985). Each plant must be assessed for the degree to which its chemical profile falls within some acceptable range, and for qualities such as water content, size, and age, which may be correlated with larval growth success (Feeny *et al.* 1983).

Larvae should also be able to recognize and assess new host plants after completely defoliating or falling from their first host plant. Dietary experience can influence the ability of insects to taste plant chemicals that signal the suitability or unsuitability of a particular host (Jermy *et al.* 1982). The theory of larval memory, commonly known as Hopkins’ host selection principle (HHSP) postulates that the adult females of phytophagous insects will prefer to feed or oviposit on the same plant species upon which they themselves developed as larvae (Hopkins, 1917 cited in Barron, 2001). The memory of the feeding substrate is stored in the central nervous system (CNS), and transferred through metamorphosis to the adult stage.

The induction of host preferences has been examined in many insect orders such as Lepidoptera (Akhtar & Isman, 2003; Anderson *et al.* 1995; Papaj and Prokopy, 1986), Diptera (Jaenike, 1982, 1988) and Coleoptera (Rietdorf and Steidle, 2002). In most cases, previous exposure to deterrent compounds significantly reduced aversion at subsequent encounters. However, exposure gained by the imago before, during or shortly after emergence from the pupa could be influencing host preference (Jaenike, 1988). At present there is still very little convincing evidence, for pre-imaginal conditioning of host choice as suggested by HHSP (Barron, 2001).

Alternatively, the concept of chemical legacy presumes that the presence of chemicals at sensitive periods, such as adult emergence, can reduce peripheral sensitivity, causing changes in subsequent adult behaviour (Corbet, 1985). Contamination from the preimaginal environment may occur when traces of chemicals from the larval stage persist either in the haemolymph of the insect or outside the pupa (Anderson *et al.* 1995). Consequently, the effects of chemical legacy might be reduced or eliminated by washing the pupa.

The influence of larval feeding experience on subsequent oviposition and feeding behavior continues to be controversial, and HHSP and chemical legacy have yet to be clearly demonstrated or negated. In this study we explore

these concepts by looking at the effects of larval feeding experience with a complex plant latex on subsequent larval feeding behaviour, and adult oviposition behaviour. We also look for the transfer of information from adults to offspring.

The cabbage looper moth, *Trichoplusia ni* Hubner (Lepidoptera: Noctuidae) was chosen as a model insect. *T. ni* is a generalist, feeding on a relatively diverse array of host plants. It is an important pest of cruciferous plants, but also attacks other crops including lettuce, beet, peas, celery, cotton, tomatoes, ornamental plants, and many weedy species. *T. ni* is suitable for this study because it has a short life cycle (egg-adult, 24-33 days) and lays eggs singly, mostly on the lower surface of leaves.

The material used for the choice experiments was a latex from *Hoodia gordonii*, a South African milkweed (Asclepiadaceae). It was selected based on oviposition and feeding deterrent properties determined in a preliminary study with other objectives. As a New World species, *T. ni* would never encounter *H. gordonii* in nature; we have used these two species strictly as a model system to study the influence of plant chemistry on insect behavior. The main objectives of our present study were to determine: (i) the effects of *H. gordonii* on larval feeding behavior and adult oviposition behaviour; (ii) if larval experience with the *H. gordonii* latex affects subsequent larval feeding and adult oviposition behaviors; and (iii) if the effects of larval experience carry over to the next generation. The research presented here provides insight into the role of HHSP and chemical legacy in host preference of lepidopterous insects.

Materials and methods

Plant Material

Cabbage plants (*Brassica oleraceae* var. Stonehead) were routinely grown in plastic pots with a mixture of sandy loam soil and peat moss (4:1) in a greenhouse at the University of British Columbia, Vancouver, BC, Canada. Leaves were collected from cabbage plants that were 5-6 weeks old.

Test material

Experiments involved a complex plant latex from the African milkweed, *Hoodia gordonii* (Asclepiadaceae), a gift from Phytopharm plc (Cambridge, UK). The plant latex (lot LB99/0020) consisted of spray-dried sap from the sliced and pressed stems of the plant grown in screen cages in South Africa. Methanol-dichloromethane (MeOH:CH₂Cl₂, 2:1, v/v) was used as a carrier.

Test Insects

Trichoplusia ni moths were obtained from a laboratory colony maintained for > 60 generations. Colonies were reared under a 16:8 LD photoperiod at room temperature (19-25° C). Larvae were reared on Velvetbean Caterpillar Diet #F9796 [Bio-Serv Inc. (Frenchtown, NJ.)]. For every 300 g of diet, 30 g of Vanderzant's vitamins and 20 g alfalfa meal were added.

General Procedure

In each experiment there were three groups of insects: naïve, experienced and the offspring of experienced. The experienced groups were reared on an artificial diet treated with the *H. gordonii* latex at 1000 ppm. Naïve insects and the offspring of experienced insects were reared on untreated artificial diet.

The concentration used for rearing was based on preliminary experiments, and did not cause significant larval growth inhibition (unpublished data). For every 200 g of diet, 0.2 g of *H. gordonii* latex was dissolved in 6 ml of carrier solvent, and applied to 35 g of powdered diet with a micropipette. Carrier solvent alone was applied to the control diet for naïve groups. After the solvent had evaporated from the powdered diet it was combined with agar (agar (g):water (ml), 1:32) that had been brought to a boil in the microwave. Fifty g of diet was poured into styrofoam cups and allowed to set. Approximately 40 neonate larvae (< 24 hours old) were placed in each styrofoam cup, and allowed to feed *ad libitum* until the bioassays were conducted. Plastic lids were placed over the cups to prevent larvae from escaping. Naïve and the offspring of experienced groups were reared under the same conditions, except that carrier solvent alone was applied to the powdered diet.

Feeding deterrence. Feeding response of fourth instar *T. ni* larvae was measured according to the leaf disc choice bioassay described by Akhtar *et al.* (2003) to Larvae were given the choice of feeding on two cabbage leaf discs, one treated with a *H. gordonii* solution (10 µl applied to each side with a pipettor), the other with carrier solvent alone. An experienced group (reared on a diet containing 1000 ppm *H. gordonii* from the neonate stage) was tested along with a naïve group (no prior exposure to *H. gordonii*) at 25 µg/cm² (naïve, n = 25; experienced, n = 22). Subsequently, the larval offspring of experienced moths were tested along with naïve larvae at *H. gordonii* concentrations of 25, 50, 100 and 200 µg/cm² (naïve, n = 18-25; experienced, n = 20-25). All groups of larvae were starved for four hours prior to bioassay. Bioassays were terminated when ~50% of the control disc had been eaten (normally 3-5 hours). Leaf disc areas consumed were measured via digital image analysis and feeding deterrence calculated as detailed in Akhtar *et al.* (2003).

Oviposition deterrence. Oviposition response of *T. ni* moths was measured according to the oviposition choice bioassay described by Akhtar and Isman (2003), with minor modifications. For experienced moths, the entire larval period was spent feeding on the treated diet, but pupae were removed from the diet within 24 hours of pupation. In this study, experienced and naïve pupae were subdivided into two groups, those that were rinsed with distilled water and those that were not (Fig. 1). After eclosion, male/female pairs of moths (n = 23-37 pairs) were maintained for 48 hours with a control and a treated leaf; subsequently, the numbers of eggs on each leaf were counted. The concentration used for testing was 0.25% (w/w). This value was based on the lowest concentration that elicited 50% oviposition deterrence (OD₅₀) relative to controls, as determined in preliminary experiments.

Results

Feeding deterrence

In an experiment testing the antifeedant effect of the latex at 25 µg/cm², experienced larvae had a mean feeding deterrence of only 11.5 ± 19.3%, whereas naïve larvae had a mean deterrence of 85.1 ± 5.7% at the same concentration. Therefore, the experienced larvae were significantly less deterred than naïve larvae under the same conditions (two sample t-test, df = 46, t = -3.85, p = 0.002).

The latex acted as an antifeedant to both naïve fourth instar larvae and to the larval offspring of experienced groups. Regression analysis was done on Sigmaplot 200 for Windows Version 6.00, using the formula $y = 100 * (1 - b^x)$ with the constraint $0 < b < 1$ (naïve, $y = 100 * (1 - 0.9844^x)$, $R^2 = 0.9309$; offspring of experienced, $y = 100 * (1 - 0.9857^x)$, $R^2 = 0.9034$) (Fig. 2). In this experiment, both the naïve cohort and the larval offspring of the experienced group exhibited low deterrence to the *H. gordonii* latex at 25 µg/cm², which is equivalent to 986 ppm, measured relative

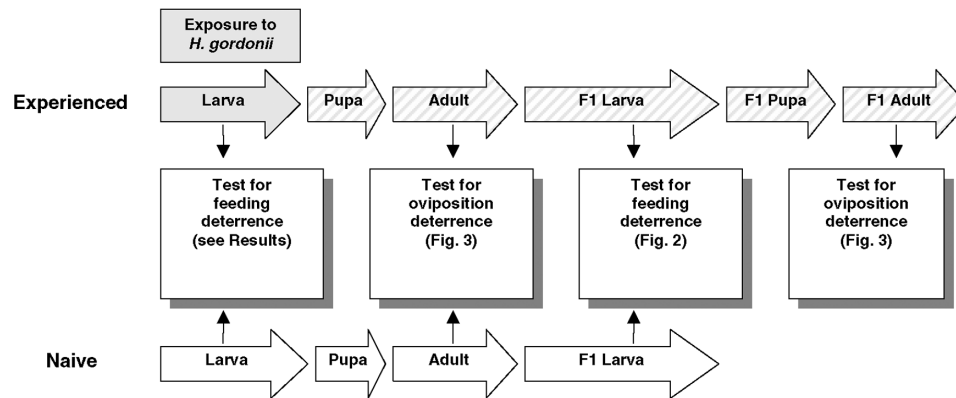


Fig. 1 Flow chart indicating insect stages tested for feeding and oviposition deterrence.

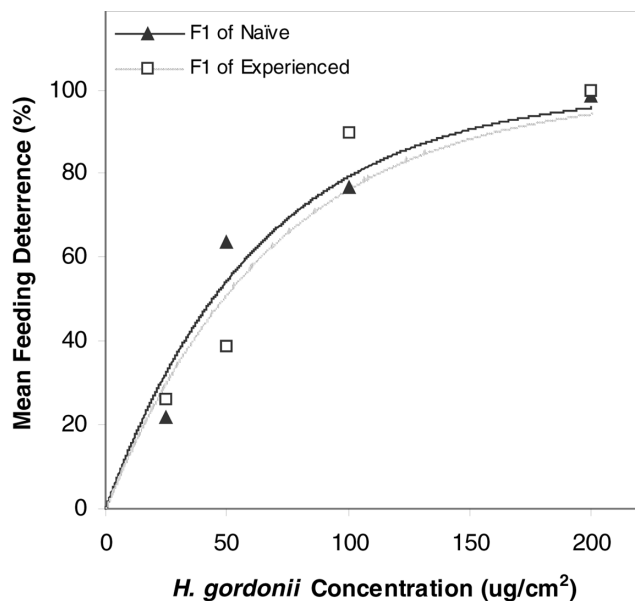


Fig. 2 Feeding responses of naïve and the offspring of experienced groups of fourth instar *T. ni* larvae to a *H. gordonii* latex at 25, 50, 100, 200 $\mu\text{g}/\text{cm}^2$ (naïve, $n = 18, 18, 25$ and 19; offspring of experienced, $n = 20, 24, 24$ and 25 for the respective concentrations). Prior to the experiment larvae were starved for 4 hours.

to the fresh weight of leaf material. This is concordant with the results of preliminary studies that showed no effect of 1000 ppm *H. gordonii* latex on neonate larval growth. However, nearly complete feeding deterrence by the *H. gordonii* latex was observed at 200 $\mu\text{g}/\text{cm}^2$ (equivalent to ~8000 ppm or 0.8% fwt).

Oviposition deterrence

A two-way ANOVA on the oviposition deterrence exhibited by naïve, experienced and the offspring of experienced groups of moths to treated leaves showed that there was no mean effect of the rinsing treatment ($F[1,154] = 0.812$, $p = 0.369$) (Figure 3). Larval experience did have a mean effect on the oviposition response of *T. ni* moths to the *H. gordonii* latex ($F[2, 153] = 21.614$, $p < 0.001$). Naïve

moths were more deterred by the latex than experienced moths (Tukey's test, $p < 0.001$). The effect of experience was so dramatic that overall, experienced moths preferred to oviposit on leaves treated with *H. gordonii* latex. The offspring (F_1) of experienced moths however, were deterred from laying eggs on cabbage leaves treated with the latex, unlike the response of their experienced parents. The oviposition deterrence response of the F_1 moths was not statistically different from that of the naïve group (Tukey's test, $p = 0.819$).

Discussion

This study illustrates how larval experience with a complex plant latex can determine insect host preference. Our results show that naïve groups of *T. ni* moths are deterred to feed and oviposit on cabbage leaves treated with the *H. gordonii* latex, and that larval feeding experience can lessen or reverse this deterrence. The modified behavioral preferences exhibited by experienced parents were not observed in the next generation, the members of which behaved like the naïve insects.

Newly hatched larvae that were reared on a diet with the *H. gordonii* latex (1000 ppm), showed no growth inhibition relative to larvae reared on a control diet, and reproduced normally (unpublished data). The absence of any observed physiological effects to *T. ni* larvae and moths from ingesting the *H. gordonii* latex suggests that there are no substances that are toxic to the moth, even though older *T. ni* were deterred to feed and oviposit by the complex plant latex, when it was applied topically to one of the moth's normal host plants (i.e. cabbage). This suggests that *T. ni* is not able to rapidly determine whether certain secondary plant chemicals are noxious or innocuous.

The relative advantage of learned host preference is maximized in situations where the availability of a host species is constant within generations, but varies unpredictably between generations (Papaj and Prokopy, 1986). Such is the case for the cabbage looper moth, a common agricultural pest with a short generation time. Consequently, learned host preference is expected. In this study, larval feeding experience with *H. gordonii* latex changed subsequent feeding and oviposition responses of *T. ni* to the latex, providing support for the Hopkins host selection principal.

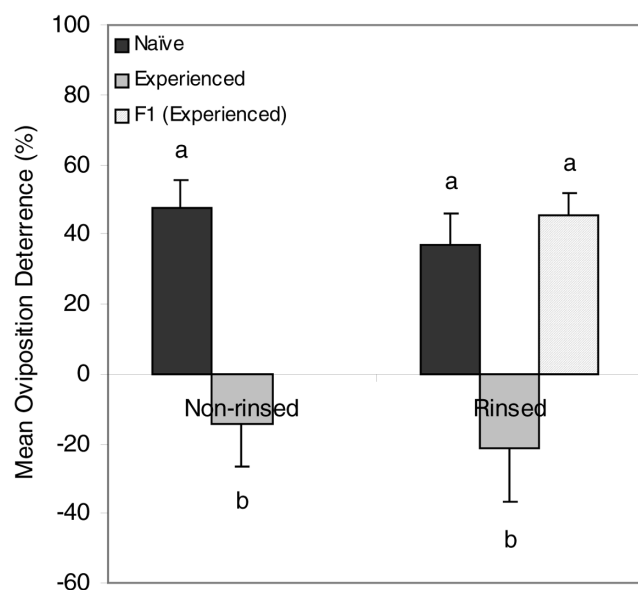


Fig. 3 Oviposition responses of naïve, experienced and the offspring of experienced groups of *T. ni* moths to a *H. gordonii* latex at 0.25% (naïve/rinsed, $n = 33$; naïve/non-rinsed, $n = 37$; experienced/rinsed, $n = 23$; experienced/non-rinsed, $n = 31$; and offspring of experienced [rinsed], $n = 32$). Mean oviposition deterrence indices (\pm SE) marked by the same letter do not differ significantly (Tukey's test, $p < 0.05$).

Experienced larvae, reared on a diet with the *H. gordonii* latex, were not as deterred to feed on treated leaf discs as naïve larvae with no previous exposure to the plant latex. The reduced feeding deterrence exhibited by experienced larvae can be explained by habituation, as previously demonstrated in this species (Akhtar *et al.* 2003).

The evolution of holometabolous development involves the separation of the larval feeding stage from the adult reproductive stage. Despite this separation and radical reorganization of the insect body during metamorphosis, we found that experienced female moths exhibited a drastic change in behavior as a result of larval exposure to *H. gordonii* (Fig. 3). Experienced female moths preferred to lay their eggs on leaves treated with the *H. gordonii* latex over control cabbage leaves, unlike naïve moths, who were deterred to lay their eggs on treated cabbage leaves. These results corroborate the study by Akhtar and Isman (2003) in which larval experience with the plant allelochemicals *trans*-anethole or toosendanin resulted in decreased oviposition deterrence by *T. ni* and a specialist moth *Plutella xylostella*.

Chemical legacy (i.e., traces of behavior-inducing chemicals carried through the pupal stage) may have played a role in the adult behavioral change. We tried to minimize those effects by rinsing and removing the pupae from the larval environment prior to the experiments, but residues of the *H. gordonii* latex may have still been present in the insect haemolymph or outside the pupal case, particularly as the intact latex is not water-soluble. Further tests have shown that the plant allelochemical toosendanin, applied topically to pupae, has no effect on subsequent adult oviposition preference (Akhtar and Isman, unpublished data).

There was no correlation between oviposition choice by experienced adults and the growth performance of their newly hatched offspring on a diet treated with the *H. gordonii* latex (unpublished data). The F1 larvae from a treated generation showed no effect of parental experience on their own feeding or oviposition preference (Fig. 2 and 3). The possibility of selection is discounted because there was no appreciable mortality ($< 15\%$ across all treatment groups) or physiological effects on the experienced larvae.

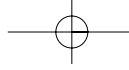
Regardless of the underlying mechanism, the observed change in oviposition preference resulting from larval experience, gives some indication of how host plant range expansion might occur in *T. ni*. If a female moth accidentally lays her eggs on a non-host species tolerated by the larvae, and these larvae survive to pupate, then the "experienced" adults might then prefer to lay their eggs on the new plant. Preference to oviposit on the same host plant on which an insect developed is one means of improving offspring survival since the fact that an adult insect survived to reproduce is testament to the suitability of a particular plant as a host.

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