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The effect of repeated exposure to feeding deterrents on their acceptability to phytophagous insects

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Abstract

In laboratory experiments last instar larvae of two oligophagous insects (*Locusta migratoria* and *Pieris brassicae*) and two polyphagous ones (*Schistocerca gregaria* and *Mamestra brassicae*) were given food treated with secondary plant substances for a limited period each day. The concentrations used were, in each case, such as to partially inhibit feeding. In several cases the experienced larvae showed gradually increasing acceptance of feeding deterrents compared with 'naive' larvae. This phenomenon has been regarded by the authors as habituation. The increased acceptance was not related to an overall augmentation of food intake. There were striking individual differences among the larvae concerning the extent of the habituation and with *S. gregaria* aversion learning has been observed. It may be that both phenomena are interrelated and occupy the extreme ends of the same response continuum. There is some indication that polyphagous species are more likely to habituate under the given experimental conditions.

Key-words: antifeedants, *Locusta migratoria, Pieris brassicae, Schistocerca gregaria, Mamestra brassicae*, habituation, aversion learning

Repeated exposure to deterrents has resulted in their increased acceptability to some insects (Strebel, 1928; Gill, 1972), but data are few and the present work describes the first experiments on how various oligo- and polyphagous insects react to such exposure. This is important in understanding natural strategies of food selection and their evolutionary changes, as well as the possibilities of either using resistant cultivars whose resistance is based on non-preference, or spraying antifeedants for pest regulation.

Materials and methods

Insect species used were final larval instars of the polyphagous *Schistocerca gregaria* Forskål (Orthoptera) and *Mamestra brassicae* L. (Lepidoptera), and the oligophagous *Locusta migratoria* L. (Orthoptera) and *Pieris brassicae* L. (Lepidoptera). All were reared in the laboratory: *M. brassicae* on a semisynthetic diet (Nagy, 1970), *P. brassicae* on cabbage, and the acridids on a mixture of wheat and hay.

The deterrent chemicals were used in concentrations which caused inhibition of feeding by about 80% in caterpillars one day after ecdysis in a 17-hour non-choice test, or 90-100% inhibition in the acridids three days after ecdysis in a 2-hour non-choice test. For the caterpillars, the deterrents were mixed into the semisynthetic diets, while for acridids they were applied to the surface of sorghum leaves or added to glass-fibre paper impregnated with sucrose solution (concentration 50 g/l). All these foods are called 'deterrent diet' (DD).

In all cases a preparatory experiment was carried out. This aimed to determine the amount consumed from the DD when the larvae were exposed to it for 17 h (caterpillars) or 19 h (acridids) daily over the instar. Newly ecdysed larvae were given the 'basic diet' (BD) for the first day. This consisted of untreated semisynthetic diet for the caterpillars or wheat for the acridids. At 16 h 00 or 15 h 00 of the same day 20 individual caterpillars or 10 acridids were transferred to DD. Next day at 09 h 00 or 10 h 00 the larvae were again given BD. In the afternoon a new cycle began, and was repeated until the larvae finished feeding at the time of the next moult. The quantity of food eaten daily through a larval instar varies and the experiment was necessary to establish what quantities of food to give stock or control treatments in the main experiment when experienced insects were eating DD.

The main experiment was carried out with insects experiencing the DD day after Day 1 as in the preparatory experiment. These are the 'experienced' (E) insects. A large number of other insects of the same age, stock (S), were fed daily with measured amounts of basic diet (BD-M), limited to the quantities appropriate for their age as determined by the preparatory experiment. For the remaining hours they received BD ad libitum. Each day a new sample of these received the DD over the same period as the experienced insects. These are the 'naive' (N) insects. Quantities of food ingested were measured. Further, for the caterpillars, control larvae having only BD-M over the period when E- and N-insects had DD were set up and sampled daily (Figure 1). The DD period was 19 h for acridids and 17 h for caterpillars. Also, for acridids the BD-M was different from the BD of wheat. It was either the sorghum leaf or sucrose-impregnated glass fibre paper. Also, the N-insects were, after their single test on DD, treated as stock insects with measured amounts of BD given during the DD period, up until ecdysis. The mean amounts of BD ingested from all stock plus returned insects gave a measure of overall BD ingested when individuals had had only one exposure to DD. Thus for acridids, the naive and control treatments are compounded.

Results

Increased intake of deterrent food In both experiments with *M. brassicae* larvae the daily intake of deterrent diet by the experienced (E) larvae increased relative to the naive (N) larvae, so that the total DD consumption of the two groups of larvae also differed significantly (Figure 2). The same applies to the strychnine experiment with *P. brassicae*. However, while the intake of DD increased in *M. brassicae* gradually during the instar, it only appeared in *P. brassicae* towards the end of the feeding period. In these three experiments with caterpillars total food consumption of experienced (E) larvae and control (C) larvae was the same, but naive larvae consumed

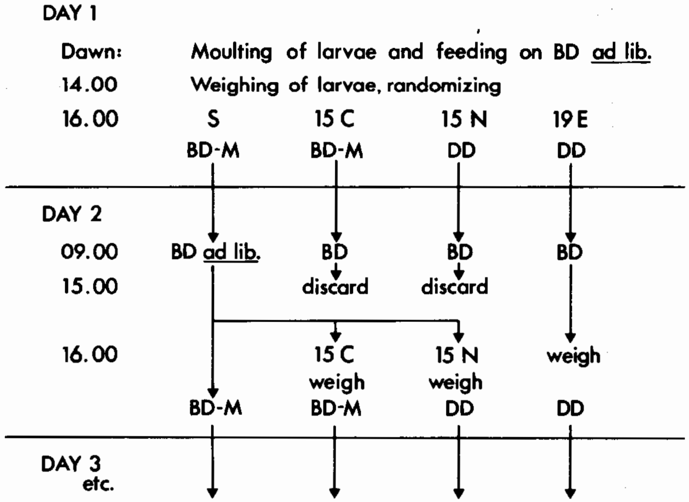

Fig. 1. Design of main experiment with caterpillars. S: stock; C: control; N: naive; and E: experienced insects. BD: basic diet; BD-M: basic diet in limited, measured amount; BD ad lib.: basic diet ad libitum; DD: deterrent diet. All food intake was monitored.

significantly less food than the other two groups. In the quinine experiment with *P. brassicae*, the control larvae consumed least (Table 1).

In both lepidopterous species, and particularly in *P. brassicae*, the experienced larvae showed striking individual differences in their ability to increase consumption of deterrent diet.

Increased ingestion of the deterrent diet was found also in *S. gregaria* when nicotine hydrogen tartrate (NHT) was applied to the surface of sorghum leaves (mass

Table 1. Total food intake (mg dry matter ± SD) of caterpillars with different deterrents and treatments.

Larval group[1]	*Mamestra brassicae*		*Pieris brassicae*	
	strychnine 0.13 mol/l	quinine 0.1 mol/l	strychnine 2.2×10^{-4} mol/l	quinine 2×10^{-4} mol/l
E-larvae (DD + BD)	575 ±69 a[2] (19)[3]	463 ± 82 a (17)	353 ± 73 a (13)	381 ± 49 a (19)
N-larvae (DD + BD)	383 ± 51 b (15)	299 ± 53 b (15)	221 ± 29 b (15)	334 ± 65ab (11)
C-larvae (BD + BD-M)	516 ± 34 a (15)	511 ± 60 a (15)	318 ± 24 a (15)	325 ± 37 b (11)

1. E: experienced; N: naive; C: control; DD: deterrent diet; BD: basic diet; BD-M: basic diet in limited, measured amount.
2. Different letters in the same column indicate significant differences at $P = 0.001$.
3. Number of replicates.

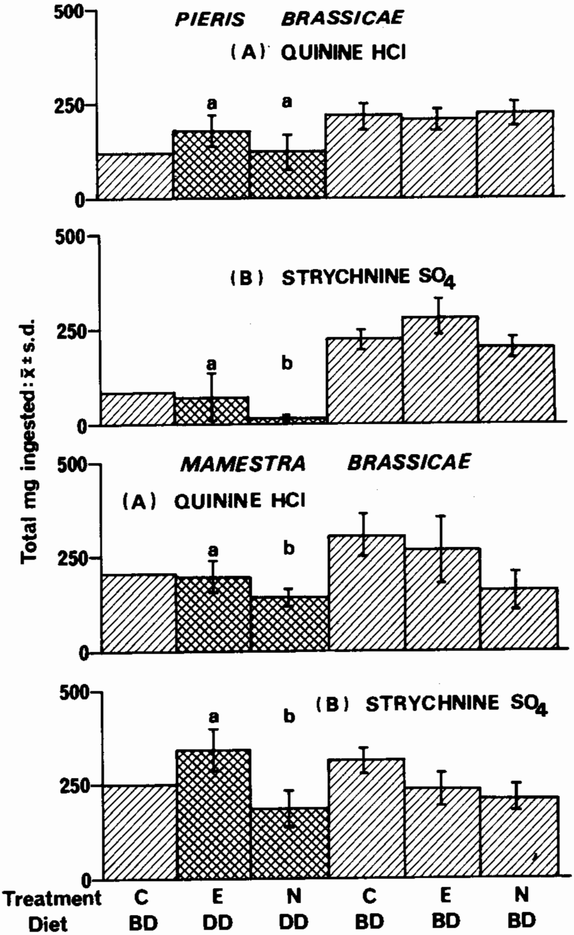

Fig. 2. Average amounts of basic and deterrent diets, respectively, consumed per larva during the whole feeding period (dry matter). For the meaning of the letters for treatment and diet see legend Figure 1. Different letters above columns indicate significant differences at $P = 0.001$. For numbers of larvae see Table 1. Note that BD was given 7 h/day (right three columns) and other diets 17 h/day (left three columns).

fraction in dry matter approximately 9 g/kg). The difference was significant for most days of the instar, with a considerable overall difference in consumption of deterrent diet but not basic diet (Figures 3 and 4).

An increase in ingestion of deterrent diet over the instar by experienced larvae of *L. migratoria* occurred when this diet was sorghum with nicotine hydrogen tartrate at mass fraction in dry matter of 3 g/kg (Figure 3). However, the increase in this case was of a quite different nature and resulted not from an obviously gradual

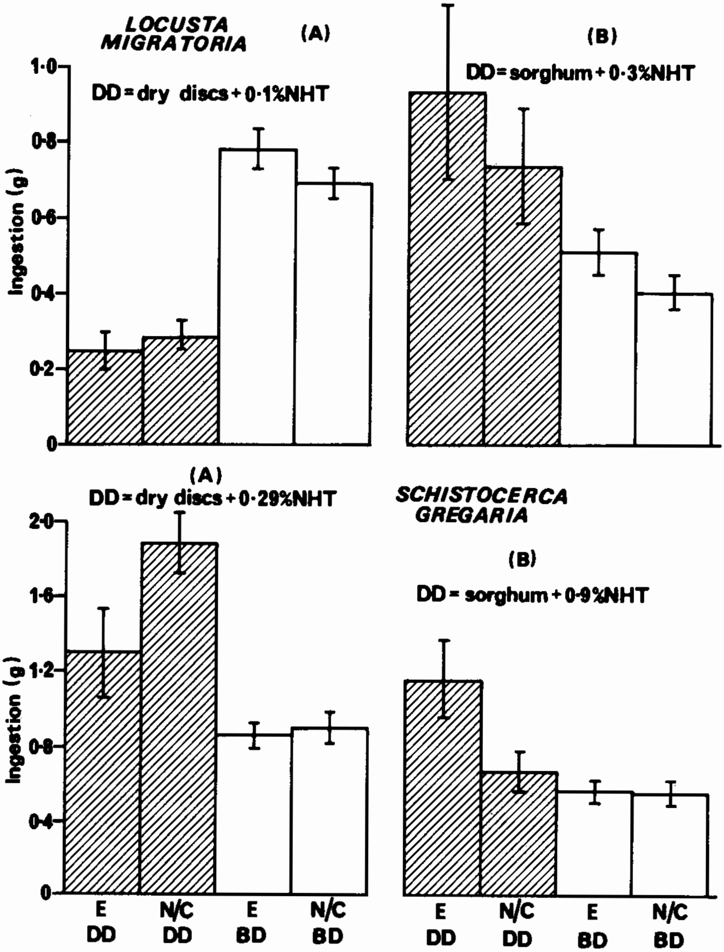

Fig. 3. Average amounts of basic and deterrent diets, respectively, consumed by acridids in four different experiments (dry matter). NHT: nicotine hydrogen tartrate. Vertical bars indicate standard deviations. For the meaning of the letters for treatment and diet see legend Figure 1. Ten insects in each treatment. Note that BD was given 5 h/day (open columns), and other diets 19 h/day (dark columns).

change, but from a prolonging of the instar through some physiological effect of the alkaloid, so that the feeding period lasted longer. Figure 4 demonstrates the contrast between increasing ingestion of deterrent diet by experienced larvae from early in the instar, and an increase in the second half of the instar associated with delayed moulting. A similar phenomenon occurred with this species when the deterrent diet was sucrose-impregnated glass fibre paper with sinigrin (mass fraction in dry matter 1 g/kg), and with *S. gregaria* having a diet of sucrose-impregnated glass fibre paper with quinine hydrochloride (mass fraction in dry matter 10 g/kg). There was no increase in mortality in the E-larvae of these experiments.

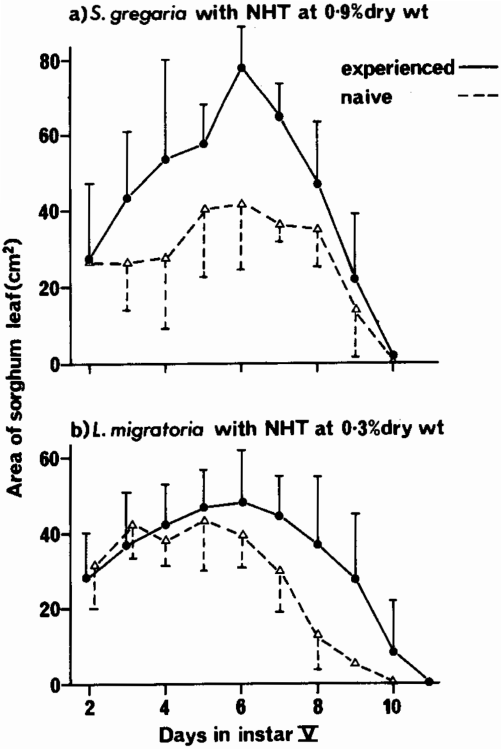

Fig. 4. Quantities of deterrent diet consumed daily over the instar by a) *Schistocerca gregaria* with nicotine hydrogen tartrate (NHT) on sorghum leaves, and b) *Locusta migratoria* with NHT on sorghum leaves. Vertical lines represent standard deviations.

Decreased intake of deterrent food This was only found with *S. gregaria*, and only when the diet was sucrose-impregnated glass fibre paper with added chemicals. Thus NHT on this substrate was eaten significantly less by the E-larvae than by the N-larvae, in marked contrast to the situation when the NHT was presented on leaf material (Figure 3). Similar results were obtained with azadirachtin at mass fraction in dry matter of 2.5 μg/kg, and linalool at mass fraction in dry matter of 0.2 g/kg (initial concentration).

No change of deterrent food intake This was the case with some deterrents tested with *L. migratoria* with sucrose-impregnated glass fibre paper plus test chemical as the deterrent. It was found with linalool, NHT and tannic acid at mass fractions of 0.1, 0.1, and 50 g/kg respectively. It was also shown with *P. brassicae* with quinine at substance concentration 0.2 mmol/l where diet consumption of E- and N-larvae differed, but only at a probability of 0.005, which was not regarded as sufficient under such experimental conditions.

Mortality caused by ingestion of deterrents This occurred in *L. migratoria* with daily ingestion of azadirachtin on glass fibre filter paper, at mass fraction in dry matter of 0.05 g/kg. Insects which ingested 5 μg or more usually died. Some individuals however, refused to eat the diet with this concentration of azadirachtin.

Discussion

The method used in all experiments aimed to minimize the differences in the degree of deprivation between the different groups of larvae by feeding the S- and C-larvae limited, measured quantities of basic diet, while the E- and N-larvae were given deterrent diet (Figure 1). This nutritional equivalence, is not a behavioural equivalence however, since the S- and C-larvae consumed the limited basic diet (BD-M) within the first few hours while the other two groups ate variably and intermittently on the deterrent diet during the whole exposure time (17 or 19 h).

In spite of this, an increased intake of deterrent diet resulted from increased exposure to it in several experiments. In three of the four experiments with caterpillars there was a significant and considerable behavioural change with no obvious physiological effects of the chemicals on them: the total food consumption of E- and C-larvae did not differ significantly (no noticeable extra cost), the final weights of the larvae were the same (no obvious deleterious effect), and there was no increase in mortality of the E-larvae. Similarly in the case of *S. gregaria* exposed to NHT on sorghum leaves, E-larvae ate considerably more of the deterrent diet with no indication of a cost in terms of extra ingestion of basic diet, time of ecdysis or final weights, which were not different in the E- and N-larvae. Since, in these experiments, the increase in tolerance to the deterrents is a consequence of the waning of the response to the inhibitory stimulus (Thompson & Spencer, 1966), it can properly be regarded as habituation. With the caterpillars, the N-larvae had a significantly reduced total food intake, as a result of a restricted feeding intensity on the basic diet for the six hours after exposure to the deterrent diet. In these cases the deterrent seemed either to invoke an inhibitory state in some neural centre coordinating food intake (Dethier et al., 1968; Jermy, 1971) or perhaps induce a general inactivity for a period. Repeated exposure apparently overcame the effect in the E-insects.

These marked increases in ingestion of deterrent diet throughout the instar are in contrast to the increased ingestion occurring at the end of the instar in some experiments in relation to a delay in ecdysis. In these cases habituation is not indicated, and presumably the costs to these insects would cause a continual decline in fitness if ingestion of the deterrent diet continued.

Decreased intake of deterrent food over a period as seen in experiments with *S. gregaria* when linalool or NHT was applied on glass fibre paper may be aversion learning, since it was not accompanied by a decrease in feeding intensity. In its appearance this response seems to be analogous to the aversion learning found by Dethier (1980) in certain caterpillars, except that in *S. gregaria* there was no noticable evidence of toxicity, which is generally believed to be associated with aversion learning. The unnatural inadequacy of the 'diet' however, is probably in some way responsible for the effect, and it must be emphasised that the same deterrent (NHT) when applied on sorghum leaves evoked habituation. It may be that both phenomena are interrelated and occupy the extreme ends of the same response continuum, the type of response to a given deterrent depending on the overall stimulus situation.

The lack of any effect of the deterrents which was found in *L. migratoria* with several chemicals tested on the glass fibre paper, as well as the fact that habituation appeared very late in *P. brassicae* with strychnine, and hardly at all with quinine, seem to indicate that oligophagous species are more rigid in their responses to deterrents.

In conclusion, the response to repeated exposure to deterrents is very variable. Although habituation has been shown, no general rules can be drawn for the understanding of food selection strategies in nature. In addition, choice situations may modify the effect. It is important to note however, that the dry inadequate diet gave results differing greatly from those obtained with the good quality leaf diet. More work is needed to investigate the different responses in relation to species or group and the overall feeding behaviour, including the type of phagism. It is also important to know whether the striking individual differences are hereditary. Progress in the practical use of antifeedants and non-preference plant resistance will depend on an understanding of these problems.

Acknowledgments

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