PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The Effect of Phagostimulant Mixtures on Deterrent Receptor (s) in Two Crucifer-Feeding Lepidopterous Species

V. D. C. Shields and B. K. Mitchell

Phil. Trans. R. Soc. Lond. B 1995 **347**, 459-464
doi: 10.1098/rstb.1995.0037

The effect of phagostimulant mixtures on deterrent receptor(s) in two crucifer-feeding lepidopterous species

V. D. C. SHIELDS[1] AND B. K. MITCHELL[2]

[1] *Department of Biology, University of Regina, Regina, Saskatchewan, Canada S4S 0A2*
[2] *Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada T6G 2E3*

SUMMARY

Sinigrin was incorporated in varying concentrations into four background mixtures. One background mixture contained potassium chloride (KCl) and no stimulatory sugar or sugar alcohol, two backgrounds contained KCl and a single sugar or sugar alcohol (sucrose or inositol, respectively), and the fourth background contained KCl and both sugar and sugar alcohol (sucrose and inositol, respectively). The lateral sinigrin-sensitive cell of *Mamestra configurata* was suppressed by phagostimulants at a low sinigrin concentration range. Electrophysiological suppression of sinigrin-sensitive cells in both the lateral and medial sinigrin-sensitive cells of *Trichoplusia ni* was effective at a low to high sinigrin concentration range. Independent of sinigrin concentration, it appeared that inositol, sucrose, and their combination, equally suppressed the lateral sinigrin-sensitive cell of *M. configurata* and a combination of both inositol and sucrose suppressed the lateral and medial sinigrin-sensitive cells of *T. ni*. There was an interaction between inositol and sucrose; inositol did not suppress or enhance the response to sucrose of the sucrose-sensitive cells in either species, but sucrose suppressed the response of the medial *M. configurata* inositol-sensitive cell to inositol. Inositol and sucrose backgrounds were effective in suppressing responses to potassium chloride in *M. configurata*, but not in *T. ni*.

These sensory-based mixture effects, all of which were suppressive, are used to propose mechanisms for the ameliorating effect of inositol and sucrose on the feeding deterrent action of sinigrin.

1. INTRODUCTION

M. configurata and *T. ni* larvae are both deterred from feeding when sinigrin is added to a moderately stimulating diet background mixture (Shields & Mitchell 1995*a*). When phagostimulants, singly (e.g. inositol, KI, sucrose, KS), or in combination (e.g. inositol and sucrose, KIS), are added to the diet background mixture, feeding increases in all cases in both species indicating that, depending on sinigrin concentration, these positive stimuli could be wholly or partly successful in suppressing the deterrent effect of sinigrin (Shields & Mitchell 1995*a*). Another study (Shields & Mitchell 1995*b*) demonstrated that sinigrin stimulated a deterrent neuron(s) in both *M. configurata* and *T. ni*.

Using dietary components, similar to those employed in previous feeding bioassay experiments (Shields & Mitchell 1995*a*), the aim of the present work is to examine the effects of stimulatory background mixtures on the response of sinigrin-sensitive (deterrent) chemosensory cells. Suppression by some phagostimulants (inositol, sucrose, and their combination) of sinigrin-sensitive cells, as well as the effect of sinigrin on sucrose-sensitive cells of *M. configurata* and *T. ni* was investigated. The effect of background mixtures on inositol, sucrose and KCl-sensitive cells was also examined.

This study provides a functional basis for the interpretation of results from a previous behavioural study (Shields & Mitchell 1995*a*) on the deterrent effect of sinigrin presented in similar backgrounds.

2. MATERIALS AND METHODS

(*a*) *Larvae and diet*

Fifth instar, 12–22 h post-moult *M. configurata* and *T. ni* larvae were obtained from an artificial diet-reared laboratory culture, as described in Shields & Mitchell (1995*a*).

(*b*) *Sensory physiology*

The electrophysiological method for recording was similar to that described in Shields & Mitchell (1995*b*).

(*c*) *Background mixture study*

In a previous behavioural study (Shields & Mitchell 1995*a*), agar, yeast and KCl served as components in every diet background mixture. Agar and yeast components were omitted from the preparation of electrophysiological background mixtures, due to the formation of a dense colloidal suspension which interfered with the free flow of test solutions in the micropipettes. In addition to the electrolyte, 60 mM sucrose (KS) was used to test the response to sucrose, and 100 mM inositol (KI) was used to test the response to inositol. A combination of the latter two solutions (KIS) was used to test the effect of inositol addition on the response to sucrose and the effect of sucrose addition on the response to inositol. A solution containing only the electrolyte (50 mM KCl) served as

a control (K) and as a moderate salt stimulus. To test consistency of response, K was applied at the beginning and end of the experiment.

Mean consumption data for each species/background combination (Shields & Mitchell 1995*a*) were consulted to determine which sinigrin concentration to use in the electrophysiological study. Sinigrin concentrations yielding significant differences in consumption, when compared to the next highest or lowest concentration, were tested electrophysiologically. Independent of background, these included: 2, 5 and 8 mM sinigrin for *M. configurata*, and 2, 5, 10 and 20 mM sinigrin for *T. ni*. A 0 mM sinigrin control for each background was also included.

Data were analysed using Gibbons (1976), Number Cruncher Statistical Software (NCSS) (1987, Dr J. Heintz, Kaysville, Utah, U.S.A.), and Statview (1986, Abacus Concepts Inc., Berkeley California, U.S.A.). To test the effect of sinigrin concentration and background on the response of the lateral (MLSin) sinigrin-sensitive cell of *M. configurata* and the lateral (TLSin) and medial (TMSin) sinigrin-sensitive cells of *T. ni* to sinigrin, the non-parametric Kruskal-Wallis one-way analysis of variance by ranks ($p \leqslant 0.05$) and Dunn's Multiple Comparison test using rank sums ($p \leqslant 0.05$) were used. The effect of background, regardless of sinigrin concentration, was tested using a parametric one-way analysis of variance ($p \leqslant 0.05$) and the two-tailed Duncan's Multiple Range test ($p \leqslant 0.05$). The effect of sinigrin addition on the response of *M. configurata* sucrose-sensitive (MLSuc) cells and *T. ni* sucrose-sensitive (TLSuc) cells was also tested; dependent on concentration (Kruskal-Wallis test, $p \leqslant 0.05$, and Dunn's test, $p \leqslant 0.05$) and independent of sinigrin concentration (parametric unpaired two-tailed *t*-test or non-parametric unpaired two-tailed Mann-Whitney *U* test; $p \leqslant 0.05$ in both cases). The latter test was used to test the effect of inositol addition on the response of sucrose-sensitive cells of *M. configurata* (MLSuc) and *T. ni* (TLSuc) and of sucrose addition on the response of the inositol-sensitive cell (MMInos) of *M. configurata*. The effect of background on the response of *M. configurata* lateral (MLKCl) and medial (MMKCl) and *T. ni* lateral (TLKCl) and medial (TMKCl) KCl-sensitive cells was tested using the Kruskal-Wallis test ($p \leqslant 0.05$) and Dunn's test ($p \leqslant 0.05$).

Recordings were made from 13 and 11 *M. configurata* and *T. ni* larvae, respectively, a total of 48 sensilla.

(*d*) *Analysis of neurophysiological recordings*

Records were digitized and analysed using methods described in Shields & Mitchell (1995*b*).

(*e*) *Glossary*

Background mixture: Components K, KI, KS, or KIS to which sinigrin was added. (KCl served as a component in every background mixture.)

Diet: agar, distilled water, yeast and KCl.

Diet background mixture: diet and components (K, KI, KS or KIS) to which sinigrin was added.

Components: K (50 mM potassium chloride); KI (50 mM potassium chloride and 100 mM inositol); KS (50 mM potassium chloride and 60 mM sucrose); KIS (50 mM potassium chloride, 100 mM inositol and 60 mM sucrose).

0 mM sinigrin control: electrode containing only KCl.

MLSin: *Mamestra* lateral sinigrin-sensitive cell.
MMInos: *Mamestra* medial inositol-sensitive cell.
MLSuc: *Mamestra* lateral sucrose-sensitive cell.
MLKCl: *Mamestra* lateral salt-sensitive cell.
MMKCl: *Mamestra* medial salt-sensitive cell.
TLSin: *Trichoplusia* lateral sinigrin-sensitive cell.
TMSin: *Trichoplusia* medial sinigrin-sensitive cell.
TLSuc: *Trichoplusia* lateral sucrose-sensitive cell.
TLKCl: *Trichoplusia* lateral salt-sensitive cell.
TMKCl: *Trichoplusia* medial salt-sensitive cell.

3. RESULTS

(*a*) *Effect of sinigrin and background mixtures on the response of sinigrin-sensitive cells of* M. configurata *and* T. ni

(i) M. configurata (*MLSin cell*)

Sinigrin evoked a robust, long-lived, phasic-tonic response from a cell in the lateral styloconic sensillum of *M. configurata* (MLSin) (Shields & Mitchell 1995*b*). At 2 mM sinigrin, inositol (KI) suppressed the response of the MLSin cell to sinigrin (table 1*a*), but suppression was not observed at 5 and 8 mM sinigrin. Sucrose (KS) and inositol and sucrose (KIS) did not significantly suppress the response of the MLSin cell to sinigrin.

(ii) T. ni (*TLSin and TMSin cells*)

Sinigrin evoked a robust, long-lived response from a cell in the lateral styloconic sensillum of *T. ni* (TLSin) (Shields & Mitchell 1995*b*). At 2, 5 and 10 mM sinigrin, sucrose (KS) and inositol and sucrose (KIS) or inositol and sucrose (KIS) suppressed the response of the TLSin cell (table 1*b*). No suppressive effect due to these phagostimulants was observed at 20 mM sinigrin. Inositol and sucrose (KIS) did not act additively to suppress the response of the TLSin cell to sinigrin.

A cell in the medial styloconic sensillum of *T. ni* (TMSin) responded strongly to sinigrin but adapted quickly (Shields & Mitchell 1995*b*). No suppressive effect due to phagostimulants was observed until 10 mM sinigrin. At this concentration, inositol and sucrose (KIS) best suppressed the response of the TMSin cell (table 1*c*). Inositol and sucrose (KIS) did not act additively to suppress the response of this cell to sinigrin.

The above data were combined to summarize the background mixture suppression phenomenon, independent of sinigrin concentration (figure 1). KI, KS and KIS were similarly effective in suppressing the MLSin cell of *M. configurata* (figure 1*a*). In *T. ni*, the TLSin cell was increasingly suppressed by these background mixtures in the order KI < KS < KIS (figure 1*b*), and the TMSin cell, in the order KI = KS < KIS (figure 1*c*).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Table 1. *Mean response of the lateral styloconic sinigrin-sensitive cell of* Mamestra configurata (*MLSin*) *and* Trichoplusia ni (*TLSin*) *and the medial styloconic sinigrin-sensitive cell of* Trichoplusia ni (*TMSin*) *to sinigrin concentrations in four background mixtures*

(Data represents the mean impulse rate ± standard error for impulses between 100–1100 ms of each stimulation. $n = 11$ and 13 lateral and medial styloconic sensilla for *Mamestra configurata* and *Trichoplusia ni*, respectively. K, 50 mmol l^{-1} potassium chloride; KI, K + 100 mmol l^{-1} inositol; KS, K + 60 mmol l^{-1} sucrose; KIS, K + inositol + sucrose. — indicates data is not available.)

sinigrin concentration	background mixture			
(mmol^{-1})	K	KI	KS	KIS
(*a*) *Mamestra configurata* (MLSin)				
2	97.1 ± 10.7^{a}	26.2 ± 10.1^{b}	66.4 ± 14.6^{a,b}	67.8 ± 15.1^{a,b}
5	81.4 ± 18.4^{a}	73.4 ± 9.3^{a}	63.6 ± 8.1^{a}	73.5 ± 9.6^{a}
8	106.1 ± 13.0^{a}	72.3 ± 13.1^{a}	76.6 ± 7.3^{a}	60.5 ± 11.6^{a}
(*b*) *Trichoplusia ni* (TLSin)				
2	127.7 ± 8.6^{a}	102.5 ± 7.7^{a,b}	87.4 ± 7.4^{b}	77.9 ± 8.7^{b}
5	132.8 ± 8.8^{a}	116.8 ± 7.0^{a,b}	79.4 ± 10.3^{b,c}	16.1 ± 11.4^{c}
10	122.1 ± 10.7^{a}	106.6 ± 9.1^{a,b}	98.7 ± 2.5^{a,b}	83.0 ± 6.4^{b}
20	111.1 ± 11.7^{a}	99.2 ± 10.5^{a}	83.2 ± 15.1^{a}	82.4 ± 13.9^{a}
(*c*) *Trichoplusia ni* (TMSin)				
2	44.2 ± 13.3^{a}	6.5 ± 2.0^{a}	5.0 ± 3.4^{a}	5.8 ± 3.3^{a}
5	53.6 ± 12.7^{a}	45.0 ± 10.0^{a}	37.6 ± 10^{a}	—
10	83.3 ± 11.2^{a}	49.6 ± 7.7^{a,b}	49.2 ± 9.7^{a,b}	32.3 ± 7.0^{b}
20	70.2 ± 10.1^{a}	57.5 ± 10.0^{a}	46.4 ± 10.7^{a}	37.7 ± 6.7^{a}

^{a,b,c} Reading across rows only, mean responses are significantly different at $p \leqslant 0.05$ (Kruskal–Wallis and Dunn's multiple comparison tests).

(*b*) *Effect of sinigrin on the response of sugar-sensitive cells of* M. configurata *and* T. ni

The effect of sinigrin on the response of the sucrose-sensitive cell (MLSuc) of *M. configurata* was tested at various sinigrin concentrations in a KS background. The mean and standard error of response to KS was 80.0 ± 57.1. Means and standard errors of response to KS in the presence of 2, 5 and 8 mM sinigrin were 66.4 ± 54.6, 63.6 ± 31.3 and 75.6 ± 30.0, respectively. Similarly, the sucrose-sensitive cell (TLSuc) of *T. ni* was tested at various sinigrin concentrations. The mean and standard error of response to KS was 84.2 ± 60.79. Means and standard errors of response to KS in the presence of 2, 5, 10 and 20 mM sinigrin were 87.4 ± 24.5, 79.4 ± 34.1, 99.0 ± 8.80 and 83.2 ± 47.6, respectively. Sinigrin did not have a significant effect on either of these sucrose-sensitive cells.

When the above data were combined to summarize the effect of sinigrin on these sucrose-sensitive cells, independent of concentration, means and standard errors of the MLSuc cell, in the absence (KS only) and presence of sinigrin (KS + sinigrin), were 80.0 ± 57.1 and 68.9 ± 38.9, respectively. Similarly, means and standard errors of the TLSuc cell, in the absence (KS only) and presence of sinigrin (KS + sinigrin), were 84.2 ± 60.8 and 87.5 ± 31.0, respectively. Sinigrin did not have a significant effect on either of the sugar-sensitive cells.

(*c*) *Interactions of sucrose and inositol on sucrose- and inositol-sensitive cells of* M. configurata *and* T. ni

The sucrose-sensitive cell in the lateral styloconic sensillum of *M. configurata* (MLSuc) usually responded to sucrose with a robust, relatively short-lived response (Shields & Mitchell 1995*b*). This was also the case for the sucrose-sensitive cell in the lateral styloconic sensillum of *T. ni* (TLSuc) (Shields & Mitchell 1995*b*). The KIS background mixture was used to test the effect of inositol on the response of sucrose-sensitive cells. The sucrose-sensitive cell of *M. configurata* (MLSuc) could easily be identified as the inositol-sensitive cell (MMInos) was not housed in the same sensillum. Because neither of the sensilla of *T. ni* contained a cell that responded to inositol, the sucrose-sensitive cell (TLSuc) could also be identified unambiguously in the record. For both cells, the response to KIS was not significantly different from that to KS. Thus, inositol did not interfere with the responses to sucrose. Means and standard errors for responses to KS and KIS were 80.0 ± 10.3 and 81.8 ± 8.32, respectively, for *M. configurata*, and 84.2 ± 16.8 and 79.5 ± 16.6, respectively, for *T. ni*.

The inositol-sensitive cell in the medial sensillum of *M. configurata* (MMInos) gave a short-lived response with very large spike amplitude when stimulated with the KI background mixture (Shields & Mitchell 1995*b*). The KIS background mixture was used to test the effect of sucrose on the response of the inositol-sensitive cell. The response of the inositol-sensitive cell to KIS was significantly different from that to KI, showing that sucrose interfered with the response to inositol. Means and standard errors for responses to KI and KIS were 131.7 ± 4.97 and 118.2 ± 5.21, respectively.

Sucrose (KS) and inositol and sucrose (KIS) were effective in suppressing the response to KCl in the lateral sensillum of *M. configurata*: 8.7 ± 0.9 (K) to 4.2 ± 1.5 (KS) and 1.2 ± 0.6 (KIS). The combination of inositol and sucrose (KIS) was especially effective in

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

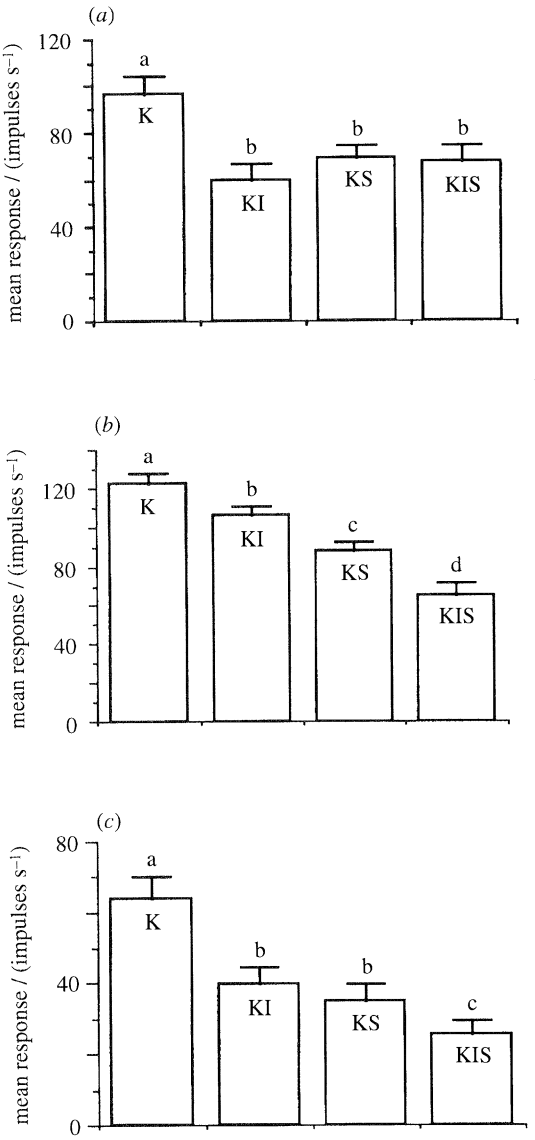

Figure 1. (*a*) Mean response from the sinigrin-sensitive cell in the lateral sensillum of *Mamestra configurata* (MLSin), independent of sinigrin concentration, in four background mixtures containing potassium chloride (K), potassium chloride/inositol (KI), potassium chloride/sucrose (KS) and potassium chloride/inositol/sucrose (KIS). Mean responses followed by different letters are significantly different at $p \leqslant 0.05$. Data analysed using one-way analysis of variance and Duncan's Multiple Range test. Each point represents means for 13 larvae (cells). Error bars represent the standard errors of the means. (*b*) Mean response from the sinigrin-sensitive cell in the lateral sensillum of *Trichoplusia ni* (TLSin), independent of sinigrin concentration, in four background mixtures, as in (*a*). Each point represents means for 11 larvae (cells). Data analysed as in (*a*). Error bars represent the standard errors of the means. (*c*) Mean response from the sinigrin-sensitive cell in the medial sensillum of *Trichoplusia ni* (TMSin), independent of sinigrin concentration, in four background mixtures, as in (*b*). Each point represents means for 11 larvae (cells). Data analysed as in (*a*). Error bars represent the standard errors of the means.

suppressing the response to KCl in this sensillum. Inositol (KI) and the combination of inositol and sucrose (KIS) were particularly effective in suppressing the response to KCl in the medial sensillum: 13.9 ± 2.7 (K) to 1.5 ± 1.0 (KI) and 1.6 ± 1.6 (KIS). Overall, the response to KCl was low in *M. configurata* compared with *T. ni*. Inositol, sucrose, or their combination had little to no effect on the response to KCl in either sensillum of *T. ni*.

4. DISCUSSION

(*a*) *Comparative action of phagostimulants on sinigrin-sensitive cells and vice-versa*

In general, inositol (KI), sucrose (KS), and their combination (KIS) suppressed the electrophysiological response to sinigrin in MLSin (*M. configurata*), TLSin and TMSin (*T. ni*) cells. This was most clearly seen by analysing the effect of sinigrin in the four background mixtures, independent of sinigrin concentration (figure 1). To our knowledge, this is the first unequivocal demonstration of suppression of a response to a feeding deterrent by a phagostimulant. The KI, KS and KIS background mixtures equally and significantly suppressed the response of the sinigrin-sensitive cell (MLSin) of *M. configurata*. In *T. ni*, the KIS background mixture was more effective than KI or KS on the two sinigrin-sensitive cells (TLSin and TMSin).

On the other hand, inhibition of phagostimulant neurons by deterrents has been well documented. Morita (1959) first demonstrated this effect for quinine on the response of the sugar-sensitive cell of *Calliphora vomitoria*. Quinine, as well as some other alkaloids, inhibits the sugar receptor of *Lymantria dispar* and *Malacosoma americanum* (Dethier 1982) and also sensilla on the galea of *Entomoscelis americana* (Mitchell & Sutcliffe 1984) and *Leptinotarsa decemlineata* (Mitchell 1987). The sesquiterpene, warburganal, suppresses the feeding response to sucrose of *Spodoptera exempta* by blocking the activity of sucrose- and inositol-sensitive cells (Ma 1977). Similar effects of warburganal were observed in the glucose- and inositol-sensitive cells of *Manduca sexta* (Frazier 1986). The anthocyanin, cyanin chloride, significantly inhibits sucrose receptors in lateral and medial styloconic sensilla of *P. brassicae* (van Loon 1990). Simmonds & Blaney (1983) found that the triterpene, azadirachtin and sucrose stimulate different neurons in the medial styloconic sensillum of *S. littoralis*, and that increasing the concentration of either compound in the stimulus mixture significantly decreases the firing rate of the other neuron. However, Simmonds *et al.* (1990) later demonstrated that increasing the sugar concentration did not necessarily decrease the firing rate of an alkaloid-sensitive cell, whereas increasing the alkaloid concentration did decrease the firing rate of the sucrose-sensitive cell. In our study, sinigrin did not significantly inhibit the sucrose-sensitive cells of either *M. configurata* or *T. ni*.

(*b*) *Correlation of sensory physiology with behaviour*

Dethier (1982) concluded that all foods contain both positive and negative factors, stimulating or deterring feeding, respectively, and that palatability of a food is a function of the ratio of positive and negative factors. In the present work, it is plausible that significant suppression of the deterrent cell(s) by the presence of one or more phagostimulants led to positive factors

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Table 2. *Behavioural and electrophysiological comparisons of the action of phagostimulants on the response of sinigrin-sensitive cell(s) of* Mamestra configurata *and* Trichoplusia ni.

insect	sinigrin concentration mM	ameliorating effects on behaviour	cells active (excluding KCl) medial	cells active (excluding KCl) lateral	suppression of sinigrin-sensitive cells by phagostimulants, independent of sinigrin concentration	styloconic sensillum housing sinigrin-sensitive cell
Mamestra configurata	1–8	K < KI ⩽ KS < KIS	inositol	sinigrin, sucrose	K < KI = KS = KIS	lateral
Trichoplusia ni	1–4	K < KI < KS < KIS	sinigrin	sinigrin, sucrose	K < KI < KS < KIS K < KI = KS < KIS	lateral medial
Trichoplusia ni	⩾ 5, < 20	K < KI < KS = KIS	sinigrin	sinigrin, sucrose	K < KI < KS < KIS K < KI = KS < KIS	lateral medial

(phagostimulants) outweighing negative ones (deterrence caused by sinigrin) in the central nervous system. This in turn resulted in the initiation or continuation of feeding. This was strongly suggested in the following behavioural results: (i) sinigrin, when presented alone in the diet (K), deterred feeding in both *M. configurata* and *T. ni*; and (ii) inositol (KI), sucrose (KS), or their combination (KIS), all significantly ameliorated the deterrent effect of sinigrin resulting in increased feeding in both species (Shields & Mitchell 1995*a*). These phagostimulants maintained normal feeding when combined with low sinigrin concentrations, however, at medium to high sinigrin concentrations, the compensatory action of the phagostimulant(s) was overridden by the deterrent, leading to a marked decrease in feeding in both species (Shields & Mitchell 1995*a*).

Electrophysiological data revealed a suppression of deterrent cell activity when sinigrin was combined with one or more phagostimulants. This suppression occurred in both *M. configurata* and *T. ni* and was in agreement with the behavioural results (Shields & Mitchell 1995*a*). Electrophysiological suppression was most effective within a defined sinigrin concentration range in *M. configurata*, for the MLSin (⩾ 2, < 5 mM) cell, and *T. ni*, for the TLSin (⩾ 2, < 20 mM) and TMSin (> 5, < 20 mM) cells (table 1). Phagostimulants were more effective in suppressing the firing rate of deterrent cells of *T. ni* than of *M. configurata*. This also correlates with behavioural results (Shields & Mitchell 1995*a*) which showed that the phagostimulants ameliorated the deterrent effect of sinigrin to a greater extent in *T. ni* than in *M. configurata*. For example, maximum feeding inhibition using KI, KS, or KIS diet background mixtures, always occurred at sinigrin concentrations that were at least twice as high in *T. ni* than in *M. configurata*.

In *M. configurata*, the KIS diet background mixture afforded maximum protection against the deterrent effect of sinigrin (KI ⩽ KS < KIS) over a wide sinigrin concentration range (1–8 mM) and, therefore, the highest mean consumption was observed when this diet background was used (table 2). In electrophysiological terms, however, KI, KS and KIS all equally suppressed (KI = KS = KIS) the sinigrin-sensitive cell (MLSin), independent of sinigrin concentration (table 2 and figure 1). The greater behavioural effectiveness of the KIS diet background mixture could be attributed to the stimulation of both inositol and sucrose-sensitive cells, an additional factor in ameliorating deterrence.

In *T. ni*, behavioural amelioration of deterrency by phagostimulants was observed over both low (1–4 mM) and medium to high (⩾ 5, < 20 mM) sinigrin concentration ranges (table 2). Behaviourally, over the low concentration range, the KIS diet background mixture was most effective in ameliorating the deterrent effect of sinigrin (KI < KS < KIS). In electrophysiological terms, KIS also most effectively suppressed the lateral (TLSin) (KI < KS < KIS) and medial (TMSin) (KI = KS < KIS) sinigrin-sensitive cells, independent of sinigrin concentration (table 2 and figure 1). Behaviourally, over the medium to high sinigrin concentration range, KS and KIS diet background mixtures were equally effective (KI < KS = KIS) (table 2). The ranking based on electrophysiology, independent of sinigrin concentration for both lateral and medial sinigrin-sensitive cells, was, however, KI < KS < KIS and KI = KS < KIS, respectively (table 2 and figure 1). Based on electrophysiology, the combination of inositol and sucrose (KIS) most effectively suppressed the sinigrin sensitive cell in each sensillum (table 2). The correlation between electrophysiological and behavioural results was clear over the low sinigrin concentration range. It is not clear why the larger suppression of the sinigrin-sensitive cells by KIS did not lead to greater behavioural amelioration of deterrency over the medium to high sinigrin concentration range.

From the behavioural and electrophysiological observations in both insect species, it can generally be concluded that increased feeding on sinigrin-treated disks, when inositol and/or sucrose were added (or in other words, the greater suppression of deterrence), was mediated by a combination of (i) inositol and/or sucrose phagostimulant cells being 'turned on', and (ii) the suppression of the deterrent cell response by these same phagostimulants.

We thank Dr B. A. Keddie, Department of Biological Sciences, University of Alberta, Edmonton, Alberta, for providing us with the *M. configurata* and *T. ni* larvae, as well as Dr O. Morris and Mr B. Wilson from Agriculture Canada, Winnipeg, who provided us initially with *M. configurata*

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

larvae and diet. We thank Dr J. van Loon, Department of Entomology, Agricultural University, Wageningen, The Netherlands, and Dr M. Weisbart, Department of Biology, University of Regina, Regina, Saskatchewan, for critically reviewing this manuscript. V.D.C.S. thanks Dr B. McCashin, Department of Biological Sciences, Edmonton. Funds for this work were provided by a Natural Science and Engineering Research Council (NSERC) of Canada postgraduate doctoral scholarship and a financial scholarship from the Faculty of Graduate Studies and Research, University of Regina, Regina, granted to V.D.C.S., and an NSERC operating grant to B.K.M. This paper is part of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Biology, University of Regina, Regina.

REFERENCES

Dethier, V.G. 1982 Mechanism of host-plant recognition. *Entomol. exp. appl.* **31**, 49–56.

Frazier, J.L. 1986 The perception of plant allelochemicals that inhibit feeding. In *Molecular aspects of insect–plant associations* (ed. L. B. Brattsten & S. Ahmad), pp. 1–42. New York: Plenum Publishing Corp.

Gibbons, J.D. 1976 *Nonparametric methods for quantitative analysis*. Toronto: Holt, Rinehart & Winston.

van Loon, J.J.A. 1990 Chemoreception of phenolic acids and flavonoids in larvae of two species of *Pieris*. *J. comp. Physiol.* A **166**, 889–899.

Ma, W.-C. 1977 Alterations of chemoreceptor function in armyworm larvae (*Spodoptera exempta*) by a plant-derived sesquiterpenoid and by sulfhydryl reagents. *Physiol. Ent.* **2**, 199–207.

Mitchell, B.K. 1987 Interactions of alkaloids with galeal chemosensory cells of Colorado potato beetle. *J. chem. Ecol.* **13**, 2009–2022.

Mitchell, B.K. & Sutcliffe, J.F. 1984 Sensory inhibition as a mechanism of feeding deterrence: effects of three alkaloids on leaf beetle feeding. *Physiol. Ent.* **9**, 57–64.

Morita, H. 1959 Initiation of spike potentials in contact chemosensory hairs of insects. III. D.C. stimulation and generator potential of labellar chemoreceptor of *Calliphora*. *J. cell. comp. Physiol.* **54**, 189–204.

Shields, V.D.C. & Mitchell, B.K. 1995*a* Sinigrin as a feeding deterrent in two crucifer-feeding, polyphagous lepidopterous species and the effects of feeding stimulant mixtures on deterrency. *Phil. Trans. R. Soc. Lond.* B **347**, 439–446. (This volume.)

Shields, V.D.C. & Mitchell, B.K. 1995*b* Responses of maxillary styloconic receptors to stimulation by sinigrin, sucrose and inositol in two crucifer-feeding, polyphagous lepidopterous species. *Phil. Trans. R. Soc. Lond.* B **347**, 447–457. (Preceding paper.)

Simmonds, M.S.J. & Blaney, W.M. 1983 Some neurophysiological effects of azadirachtin on lepidopterous larvae and their feeding response. In *Natural pesticides from the neem tree and other tropical plants* (ed. H. Schmutterer & K. R. S. Ascher), pp. 163–180. Rauischholzhausen: Eschborn.

Simmonds, M.S.J., Blaney, W.M. & Fellows, L.E. 1990 Behavioral and electrophysiological study of antifeedant mechanisms associated with polyhydroxy alkaloids. *J. chem. Ecol.* **16**, 3167–3196.

Received 4 July 1994; accepted 12 October 1994

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES