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Responses of Maxillary Styloconic Receptors to Stimulation by Sinigrin, Sucrose and Inositol in Two Crucifer-Feeding, Polyphagous Lepidopterous Species

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Responses of maxillary styloconic receptors to stimulation by sinigrin, sucrose and inositol in two crucifer-feeding, polyphagous lepidopterous species

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SUMMARY

Chemosensory cells in the lateral and the medial styloconic sensillia on the galea of larval *Mamestra configurata* and *Trichoplusia ni* were investigated electrophysiologically. Sinigrin, sucrose, inositol and potassium chloride (KCl) were tested on both sensilla of each species. One of the four cells in both the lateral and medial sensilla of *T. ni* was sinigrin-sensitive, whereas in *M. configurata*, one cell in only the lateral sensillum was sinigrin-sensitive. The lateral sinigrin-sensitive cell of *T. ni* was eightfold more sensitive than the corresponding cell of *M. configurata*. One cell in the lateral sensillum of both species was sucrose-sensitive. The medial sensillum of *M. configurata* housed an inositol-sensitive cell but no cell sensitive to inositol was present in *T. ni*. One cell in the lateral and medial sensilla of both species was KCl-sensitive. Adaptation and dose–response data are given for the sinigrin-sensitive cells in both species. These are discussed in the context of deterrent-sensitive cells reported from other Lepidoptera. Comparative physiological and evolutionary aspects are considered and the general concepts of the lepidopteran deterrent cell is discussed.

1. INTRODUCTION

Deterrent receptors in lepidopterous larvae are thought to derive from the 'common chemical sense' or salt receptor (Dethier 1980). Deterrent cells respond in a dose-dependent manner with an increased rate of firing to many plant compounds (Frazier 1986). Thus, deterrence is thought to be somewhat non-specific with respect to chemical stimulus. Different components making up a plant may play the role of deterrent for each species of insect that feeds on it. It has been postulated that caterpillars evolved a proliferation of receptor types including the deterrent receptors, which together are sensitive to a wide variety of secondary plant compounds (Dethier 1980). A central nervous command system is thought to have evolved whereby the appropriate behaviour is associated with a particular sensory input (Dethier 1980). One view emphasizes sharply defined single cell responses (labelled lines), whereas a more holistic approach searches for patterns in ensemble firing activities (across-fibre patterning) (Schoonhoven 1987).

Recent behavioural evidence shows that sinigrin, a well-known glucosinolate from certain cruciferous plants, acts as a potent feeding deterrent for *M. configurata* and *T. ni* at concentrations above 1 mM (Shields & Mitchell 1995). Blom (1978*a*) reported that sinigrin deters feeding in *Mamestra brassicae*, but stimulates feeding in *Pieris brassicae*. Similar to sinigrin, sinalbin, another glucosinolate, also deters feeding in a dose-dependent manner starting at concentrations as low as 0.1 mM in *M. configurata* (Bodnaryk 1991). Most of the research to date dealing with the electrophysiological response of caterpillars to glucosinolates, in particular sinigrin, and to phagostimulatory compounds, such as inositol and sucrose, has focused on larvae of *P. brassicae* and *M. brassicae* (Blom 1978*a*).

The aim of the present study is to evaluate the sensory input in *M. configurata* and *T. ni* by neurophysiological examination of the sensory responses of the maxillary styloconic sensilla to the feeding deterrent, sinigrin, and to phagostimulants such as inositol and sucrose.

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).

(*b*) *Sensory physiology*

Randomly selected larvae were deprived of food 2–4 h prior to the experiments. Experiments were carried out between 10h00–20h00, with 90–150 min typically required for a single series (one preparation).

Preparation for recording involved transecting the larva at mid-thorax, inserting a blunt-tipped, saline-filled, glass micropipette and drawing the cuticle over the pipette until the tip reached the head. The pipette was pushed cephalad with sufficient force to cause the separation of the mandibles and eversion of the styloconic pegs. This pipette also served as the

indifferent electrode. Lepidopterous saline was prepared according to Liu & Ryan (1991), with minor modifications. The cut end of the animal was sealed with a minimal amount of melted bee's wax. Preparations lasted 2–4 h.

Electrophysiological recordings were obtained from the lateral and medial styloconic sensilla by the standard tip-recording technique described by Hodgson *et al.* (1955) and Frazier & Hanson (1986). A clamping pre-amplifier (George Johnson, Baltimore, Maryland; see Frazier & Hanson 1986) was used to provide high input resistance and to compensate for the transepithelial potential which was much larger than the action potentials. Pre-amplifier output was filtered below 0.1 and above 3 kHz, amplified, and recorded on magnetic tape (TEAC 22–4 multitrack recorder with Vetter 2D FM adapter). A Tektronix 5112 oscilloscope allowed real-time visualization of the recording and all recordings were accompanied by a vocal record on an audio track.

Stimulating recording electrodes (6–8 μm diameter) were pulled and filled with various solutions just prior to application. Fifty millimolar KCl (Fisher Scientific Co.) was always used in the stimulating electrodes.

(*c*) *Dose–response and adaptation*

For dose–response and adaptation experiments, stimulating solutions included various concentrations of sinigrin (United States Biochemical Corporation and Sigma Chemical Co.), 60 mM sucrose–saccharose (Anachemia), 100 mM meso-inositol (J. T. Baker Chemical Co.), as well as a combined inositol/sucrose solution. A 1 or 2 mM sinigrin test stimulus was usually applied prior to each experiment to determine if a satisfactory response from that animal (good signal-to-noise ratio) could be obtained. For dose–response experiments, lateral and medial sensilla were stimulated in succession, allowing each sensillum a minimum 2 min disadaptation time between stimulations. Sinigrin was diluted with 50 mM KCl. Solutions were usually tested in ascending concentration. To ensure a reproducible response for a particular concentration being tested, 2–3 replications of each solution per sensillum were made. Repetition was especially necessary at lower sinigrin concentrations, as at these concentrations, cell activity due to sinigrin alone was often difficult to distinguish from a response to salt. Each stimulation was approximately 2–3 s in duration. For adaptation experiments, stimulation time varied from 2 s to 12 min, depending on the stimulating compound being tested.

(*d*) *Data analysis*

Only records with a good signal-to-noise ratio were analysed. For each stimulus application, one second of the response, starting 100 ms after the onset of the contact artefact, was sampled digitally and used for analysis.

Tape recorded data were digitized off-line using a MS-DOS 80286 microcomputer fitted with a Metrabyte DAS-16 A/D card. The data were sampled at 6000 samples s^{-1} and analysed using SAPID tools (Smith *et al.* 1990) software. Owing to inter-sensillum variability, all data from each sensillum were analysed independently when assigning waveforms to spike classes. Only after the individual analyses of all sensilla were completed, were the data (waveform templates) grouped to confirm across-sensillar assignment of waveforms to spike classes. The final output file containing values for impulses s^{-1} for each cell (waveform) type in each sensillum was imported into a commercial spreadsheet (Microsoft Excel 1992, Microsoft Corporation, U.S.A) for further manipulation. Number Cruncher Statistical Software (NCSS) (1987, Dr J. Heintz, Kaysville, Utah, U.S.A.) was used for statistical analyses. Other descriptions of data analysis using SAPID tools can be found in Haley-Sperling & Mitchell (1991), Mitchell *et al.* (1990) and Smith *et al.* (1990).

(*e*) *Glossary*

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*) *Cell types and basic responses*: Mamestra configurata

A cell in the lateral styloconic sensillum of *M. configurata* responded to sinigrin (MLSin) in a positive dose-dependent manner. Figure 1*a* shows the dose–response relationship for this cell to sinigrin. The threshold concentration of sinigrin for the MLSin cell was 0.16 mM. The maximal response produced in the first second of stimulation, with 30 mM sinigrin as the stimulant, was 178 impulses s^{-1} (figure 1*a*). The shape of the action potentials produced by this cell did not change with concentration or with time. The response of the MLSin cell was highly reproducible.

The response from the MLSin cell was robust (exhibited a regular firing pattern), long-lived, and had a steep (negative) initial adaptation curve. This is shown for 8 and 20 mM sinigrin in figure 1*b*. The first 10 s of the phasic responses to these sinigrin concentrations are shown in figure 1*c* (inset). The tonic response began after about 30 s for both concentrations and, in some animals, lasted for up to 12 min. Near initial maximum responses could be recorded 5 min after a 2–3 min continuous stimulation. The MLSin cell responded with a greater impulse frequency to 20 mM sinigrin (169 impulses s^{-1}), than to 8 mM (147 impulses s^{-1}) (figure 1*b*), and the adaptation curves ran parallel to one another with the higher sinigrin concentration producing the higher response rates over the entire stimulation period. The shape of the adaptation curve for 5 mM sinigrin (not shown) resembled those for 8 and 20 mM sinigrin. The tonic

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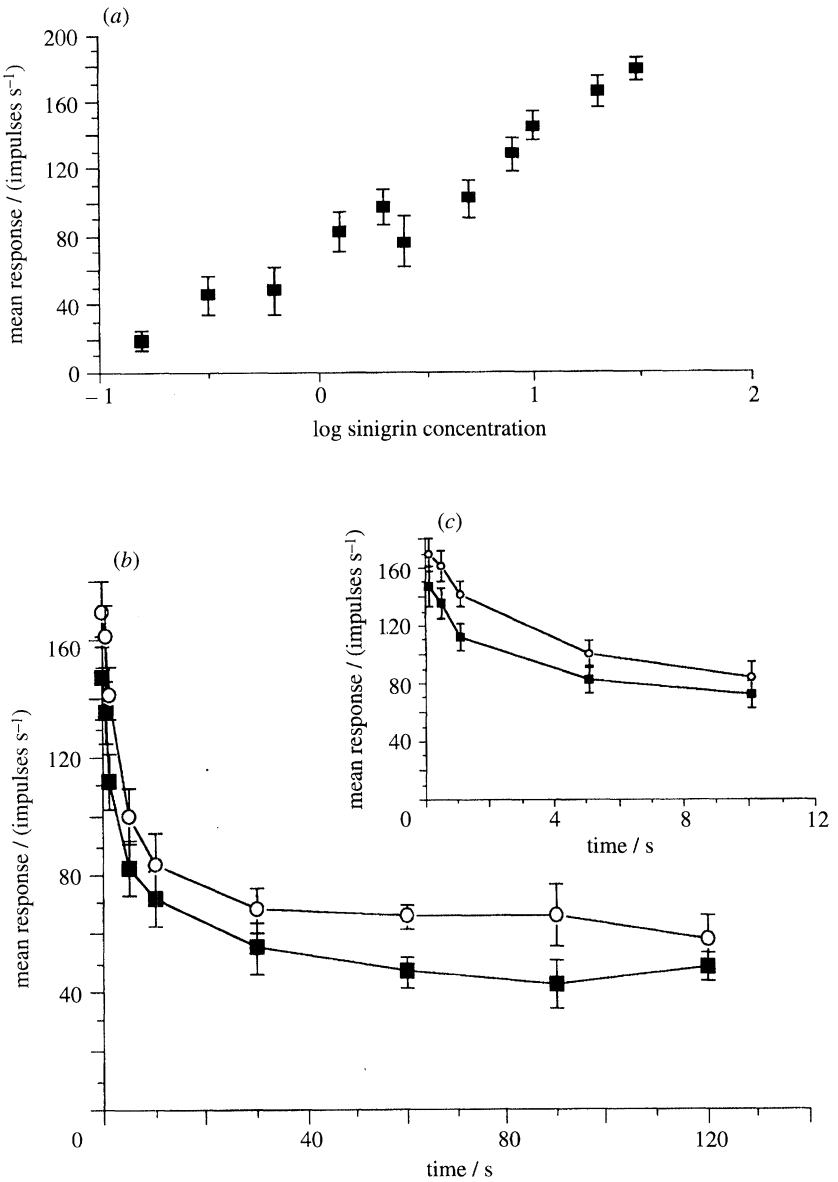

Figure 1. (*a*) Dose–response curve for the sinigrin-sensitive cell in the lateral styloconic sensillum of *Mamestra configurata* (MLSin) during stimulation with various concentrations (mM) of sinigrin. Each point represents 10–23 larvae (cells). Error bars represent the standard errors of the means. (*b*) Adaptation curves for the sinigrin-sensitive cell in the lateral styloconic sensillum of *Mamestra configurata* (MLSin) during stimulation with 8 mM (filled squares) and 20 mM (open circles) sinigrin. Each point represents means for 4–6 larvae (cells). Error bars represent the standard errors of the means. (*c*) Inset shows the first 10.1 s of the adaptation response.

response to both 8 and 20 mM sinigrin (figure 1*b*) usually continued beyond 120 s. A representative recording of the MLSin cell response to sinigrin is shown in figure 3*b*.

One cell in each of the lateral and medial styloconic sensilla of *M. configurata* responded to KCl (MLKCl and MMKCl, respectively). Both cells produced a weak response to KCl. Representative MLKCl and MMKCl recordings are shown in figure 3*a*, *d*, respectively.

Sixty millimolar sucrose evoked a response from a cell in the lateral styloconic sensillum of *M. configurata*. (MLSuc). The MLSuc cell fired with a maximum impulse frequency of 177 impulses s^{-1} with 60 mM sucrose as the stimulant (figure 2). The spike amplitude of the MLSuc cell was lower than that of the MLSin cell and the two could be easily separated using SAPID software. The tonic response commenced after about 2 s and complete adaptation (indistinguishable from the KCl background) occurred after about 3 s (figure 2). Dose–response data were not obtained for this cell. A representative recording of the MLSuc cell's response to sucrose is shown in figure 3*c*.

A cell in the medial styloconic sensillum increased its firing rate in response to 100 mM inositol (MMInos). The MMInos cell fired with a maximum impulse frequency of 148 impulses s^{-1} with 100 mM inositol as the stimulant (figure 2). The spike amplitude of the MMInos cell was greater than that of the MLSin and MLSuc cells; however, action potential amplitudes diminished over time during a single record. The phasic portion of the adaptation curve resembled that of the MLSuc cell in duration, with the tonic response beginning after about 2 s and complete adaptation

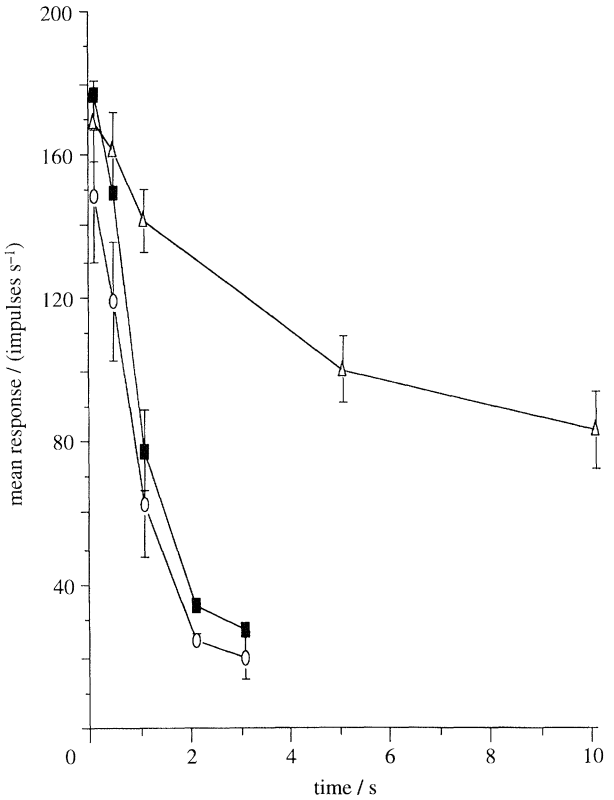

Figure 2. Adaptation curve for the sinigrin-sensitive (MLSin) and sucrose-sensitive (MLSuc) cells in the lateral styloconic sensillum during stimulation with 20 mM sinigrin (open triangles) and 60 mM sucrose (filled squares), respectively, and inositol-sensitive cell (MMInos) in the medial styloconic sensillum during stimulation with 100 mM inositol (open circles) of *Mamestra configurata*. Each point represents means for 4–6, 2 and 2–5 larvae (cells), respectively. Error bars represent the standard errors of the means.

Table 1. *Number and identity of cells and their types firing in each maxillary styloconic sensillum of* Mamestra configurata *and* Trichoplusia ni *in response to various stimulating mixtures*

(sin = sinigrin-sensitive cell; inos = inositol-sensitive cell; suc = sucrose-sensitive cell; KCl = potassium chloride-sensitive cell. All mixtures contained 50 mM KCl.)

(*a*) *Mamestra configurata*

compound and concentration/ mM	medial sensillum	lateral sensillum
50 KCl	1 salt	1 salt
100 inositol	1 inos	1 salt
60 sucrose	1 salt	1 suc
100 inositol + 60 sucrose	1 inos	1 suc
2 sinigrin	1 salt	1 sin
2 sinigrin + 100 inositol	1 inos	1 sin
2 sinigrin + 60 sucrose	1 salt	1 sin 1 suc
2 sinigrin + 100 inositol + 60 sucrose	1 inos	1 sin 1 suc

(*b*) *Trichoplusia ni*

compound and concentration/ mM	medial sensillum	lateral sensillum
50 KCl	1 salt	1 salt
100 inositol	1 salt	1 salt
60 sucrose	1 salt	1 suc
100 inositol + 60 sucrose	1 salt	1 suc
2 sinigrin	1 sin	1 sin
2 sinigrin + 100 inositol	1 sin	1 sin
2 sinigrin + 60 sucrose	1 sin	1 sin 1 suc
2 sinigrin + 100 inositol + 60 sucrose	1 sin	1 sin 1 suc

occurring after about 3 s (figure 2). A representative recording of the MMInos cell is shown in figure 3*e*. Inositol was the only one of the tested compounds that stimulated a major response in the medial sensillum of *M. configurata*. Dose–response data were not obtained for this cell. Figure 2 compares the responses of the MLSin, MLSuc and MMInos cells to sinigrin, sucrose and inositol, respectively. Table 1*a* summarizes the number of waveform types (cells) detected in *M.*

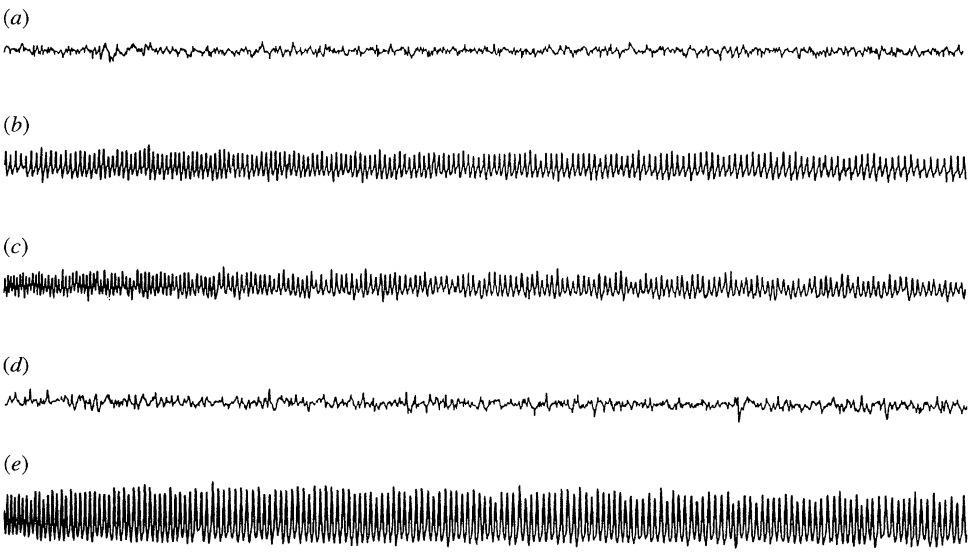

Figure 3. Representative responses from the lateral and the medial styloconic sensillia on the galea of *Mamestra configurata* fifth instar larvae in response to: (*a*) 50 mM potassium chloride; (*b*) 2 mM sinigrin; (*c*) 60 mM sucrose, from lateral sensilla; (*d*) 50 mM potassium chloride; and (*e*) 100 mM inositol, from medial sensilla. All recordings were made from the same preparation. 50 mM potassium chloride served as the electrolyte.

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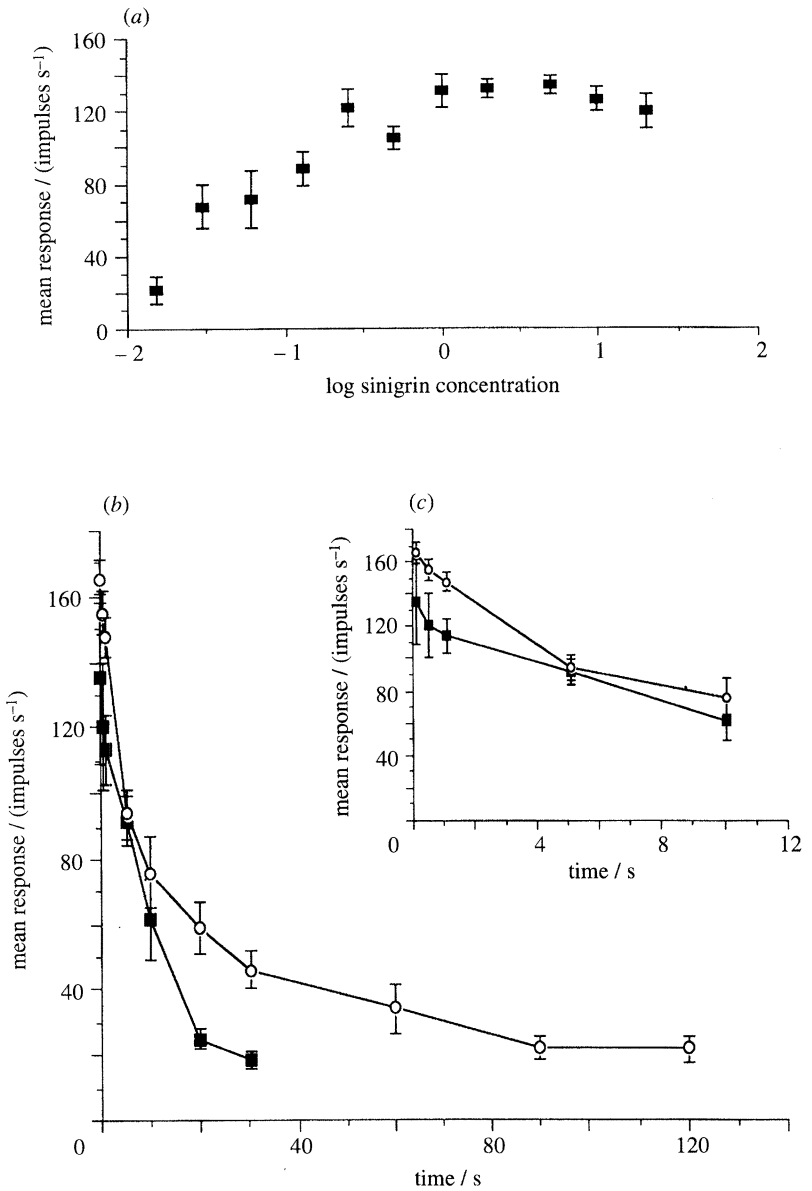

Figure 4. (*a*) Dose–response curve for the sinigrin-sensitive cell in the lateral styloconic sensillum of *Trichoplusia ni* (TLSin) during stimulation with various concentrations (mM) of sinigrin. Each point represents means for 11–22 larvae (cells). Error bars represent the standard errors of the means. (*b*) Adaptation curves for the sinigrin-sensitive cell in the lateral styloconic sensillum of *Trichoplusia ni* (TLSin) during stimulation with 2 mM (filled squares) and 20 mM (open circles) sinigrin. Each point represents means for 2–6 larvae (cells). Error bars represent the standard errors of the means. (*c*) Inset shows the first 10.1 s of the adaptation response.

configurata in response to a sample of the various stimulatory compounds used in this study.

(*b*) *Cell types and basic responses*: Trichoplusia ni

In *T. ni*, two cells, one each in the lateral and medial styloconic sensilla, responded to sinigrin (TLSin and TMSin, respectively). Both the TLSin and TMSin cells responded in a positive dose-dependent manner (figures 4*a* and 5*a*). The threshold concentration for sinigrin in TLSin and TMSin cells was approximately 0.02 mM and 0.06 mM, respectively; the maximal response produced in the first second of stimulation, with 20 mM sinigrin as a stimulant, was 134 and 74 impulses s^{-1}, respectively (figures 4*a* and 5*a*). As in *M. configurata*, the action potentials did not change in shape over time. The response from both cells was robust and highly reproducible. The TLSin sinigrin receptor(s) became saturated at 5 mM sinigrin (134 impulses s^{-1}) and the TMSin sinigrin receptor(s), at 10 mM (74 impulses s^{-1}) (table 2).

The TLSin cell response also had a steep adaptation curve when stimulated by 2 and 20 mM sinigrin (figure 4*b*). The first 10 s of the phasic response to 2 and 20 mM is shown in figure 4*c*. The tonic response commenced after about 60 s with 20 mM sinigrin as the stimulant and lasted, in most animals, for 90–120 s. After this time, the response became indistinguishable from that to KCl. The tonic response began after 20 s with 2 mM sinigrin as the stimulant and complete adaptation occurred after 30 s. The TLSin cell fired with a greater impulse frequency when 20 mM sinigrin was applied (165 impulses s^{-1}), than with 2 mM sinigrin (135 impulses s^{-1}, figure 4*b*). The adaptation curves also

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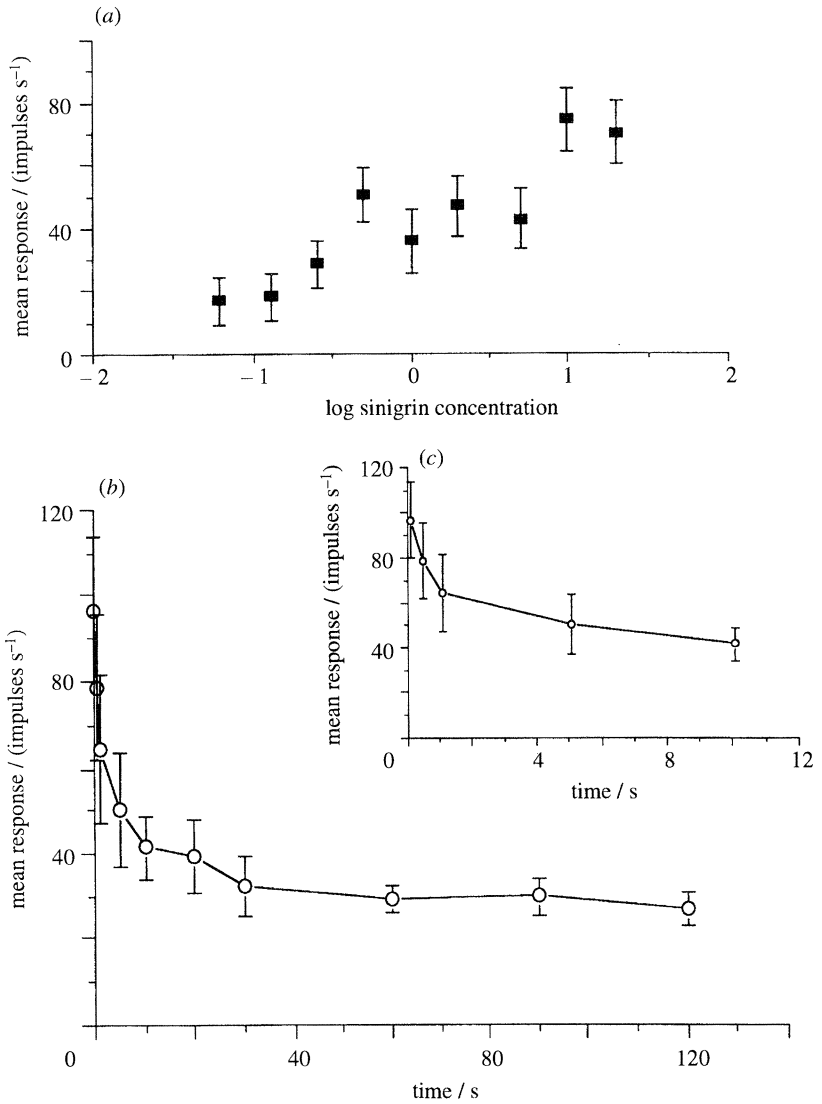

Figure 5. (*a*) Dose–response curve for the sinigrin-sensitive cell in the medial styloconic sensillum of *Trichoplusia ni* (TMSin) during stimulation with various concentrations (mM) of sinigrin. Each point represents means for 11–22 larvae (cells). Error bars represent the standard errors of the means. (*b*) Adaptation curves for the sinigrin-sensitive cell in the medial styloconic sensillum of *Trichoplusia ni* (TMSin) during stimulation with 20 mM sinigrin. Each point represents means for 3–6 larvae (cells). Error bars represent the standard errors of the means. (*c*) Inset shows the first 10.1 s of the adaptation response.

differed; 2 mM sinigrin produced a much steeper phasic response than did 20 mM. The adaptation curve for 5 mM sinigrin (not shown) resembled that of 20 mM sinigrin. The response of the TLSin cell to sinigrin was highly reproducible.

The response of the TMSin cell had a typical adaptation curve when stimulated by 20 mM sinigrin (figure 5*b*). The first 10 s of the phasic response is shown in figure 5*c*. The tonic response commenced after about 30 s and lasted in the majority of animals for approximately 120 s. The TMSin cell fired with 97 impulses s^{-1} to 20 mM sinigrin (figure 5*b*).

The responses of the TLSin and TMSin cells to 20 mM sinigrin differed not only in impulse frequency but in the duration of their phasic-tonic dynamics. In the TLSin cell, 165 impulses s^{-1} were recorded compared with 97 in the same period for the TMSin cell (figure 6). The tonic response began after approximately 60 s in the TLSin cell, whereas in the TMSin cell, it began after only 30 s (figure 6). Representative recordings of TLSin and TMSin cells are shown in figure 7*b*, *h*, respectively. Representative recordings of TLSin sensilla in response to sinigrin, in the presence of inositol, sucrose, and inositol and sucrose are shown in figure 7*c–e*, respectively.

Cells responded in both lateral and medial styloconic sensilla of *T. ni* to KCl (TLKCl and TMKCl, respectively). Both cells produced weak responses to KCl. Representative TLKCl and TMKCl recordings are shown in figure 7*a*, *g*, respectively.

One cell in the lateral styloconic sensillum responded to 60 mM sucrose (TLSuc). The TLSuc cell fired with a maximum impulse frequency of 139 impulses s^{-1} with 60 mM sucrose as the stimulant (figure 6). The tonic response in the TLSuc cell commenced after about 5 s. Complete adaptation of the TLSuc cell did not occur after 30 s (figure 6). A representative recording of the TLSuc cell's response to sucrose is shown in figure 7*f*. Dose–response data were not obtained for this cell. There was no response from either sensillum of *T. ni* to

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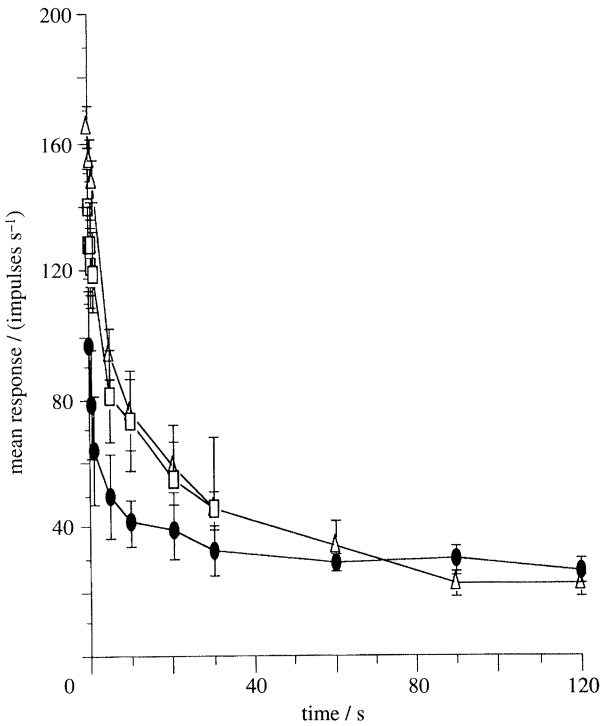

Figure 6. Adaptation curves for the sinigrin-sensitive cells in the lateral (TLSin; open triangles) and the medial (TMSin; filled circles) styloconic sensillium during stimulation with 20 mM sinigrin, and sucrose-sensitive cell (TLSuc) in the lateral styloconic sensillum during stimulation with 60 mM sucrose (open squares) of *Trichoplusia ni*. Each point represents means for 2–6, 4–8 and 3–6 larvae (cells), respectively. Error bars represent the standard errors of the means.

100 mM inositol. Figure 6 compares the responses of the TLSin and TMSin cells to sinigrin and the TLSuc cell to sucrose. Table 1*b* summarizes the number of waveform types (cells) detected in *T. ni* in response to a sample of the various stimulatory mixtures used in this study.

Table 2 summarizes dose–response parameters of the sensory response of three sinigrin-sensitive cells of *M. configurata* and *T. ni*. These parameters were derived from the dose–response data presented in figures 1, 4 and 5, and from double-reciprocal plots of these data (not shown). Table 3 summarizes the evolutionary host-plant relationships and varying characteristics of lateral and medial sensilla of *M. configurata* and *T. ni*, as well as two other crucifer-feeding insects.

4. DISCUSSION

(*a*) *Deterrent neurons*

Schoonhoven & Jermy (1977) pointed out the importance of investigating both electrophysiological and behavioural responses (both in the same concentration range and within the concentration range found within the plant) to determine whether the information processed by sensory cells caused the activation or inhibition of feeding. They concluded that feeding deterrents could act on the sensory system by stimulating specialized deterrent receptor cells or by modifying the activity of cells responding to feeding stimulants. Schoonhoven (1981) listed 24 lepidopterous species for which there is electrophysiolgical evidence for the presence of deterrent-sensitive cells. Earlier, Schoonhoven (1973) listed those chemicals which stimulate a deterrent-sensitive cell in medial sensilla of three caterpillar species, while Wieczorek (1976) listed those substances which specifically stimulated the lateral styloconic 'glycoside receptor.'

Schoonhoven (1982) suggested that feeding deterrents may alter sensory input by: (i) stimulating specific deterrent receptors; (ii) stimulating broad spectrum receptors; (iii) stimulating activity of some cells and inhibiting others, thereby changing complex and subtle codes; (iv) inhibiting specific phagostimulant receptors; or (v) evoking highly unnatural impulse patterns, often at high frequency. Blaney *et al.* (1988) stated that there was uncertainty about whether the ability of a 'deterrent' neuron to respond to a wide range of chemicals was due to it having a diverse range of receptor sites, each with its own structure–function specificity, or due to the active chemicals having common features making them able to interact with a single receptor site.

Detailed information about behavioural and electrophysiological responses of *M. brassicae* larvae to deterrents is provided in Wieczorek (1976) and Blom (1978*a*), and for *P. brassicae* in Blom (1978*a*, *b*) and Ma (1969, 1972).

(*b*) *Criteria for, and sensitivity and adaptation in, deterrent-sensitive cells*

Using *P. brassicae* as a model and the feeding deterrent, strychnine, Schoonhoven (1982) argued that deterrent neurons possess a number of unique characteristics. They generally adapt more slowly than cells which respond to phagostimulatory compounds and the tonic activity of the deterrent receptor stabilizes at a higher level than in the other cells. This 'differential adaptation' hypothesis, proposed by Schoonhoven, while broadly accurate, should be tested on each new system to determine the degree to which it might contribute to neural coding. He used the different adaptation rates to argue that the sensory code changes with time, with the result that the deterrent receptor activity gradually becomes more pronounced in the sensory message sent to the brain. Food which at the beginning of a meal may be acceptable, soon becomes unacceptable because of the more prominent share of the deterrent in the total sensory impression.

Using *P. brassicae*, Schoonhoven & Blom (1988) also determined that impulses from the deterrent cells are given a greater weight by the central nervous system; therefore, one impulse from a medial deterrent neuron neutralizes 2.5 impulses from phagostimulant-sensitive cells. Furthermore, cells signalling the presence of allelochemics usually respond to about 1000 times lower concentrations than the receptors measuring the quantity of nutrients.

Hanson & Peterson (1990), using *Manduca sexta* as a model, added three more characteristics that help to define the response of the deterrent cell: a relatively long latency period prior to the tonic response, a slow increase in spike frequency following stimulus ap-

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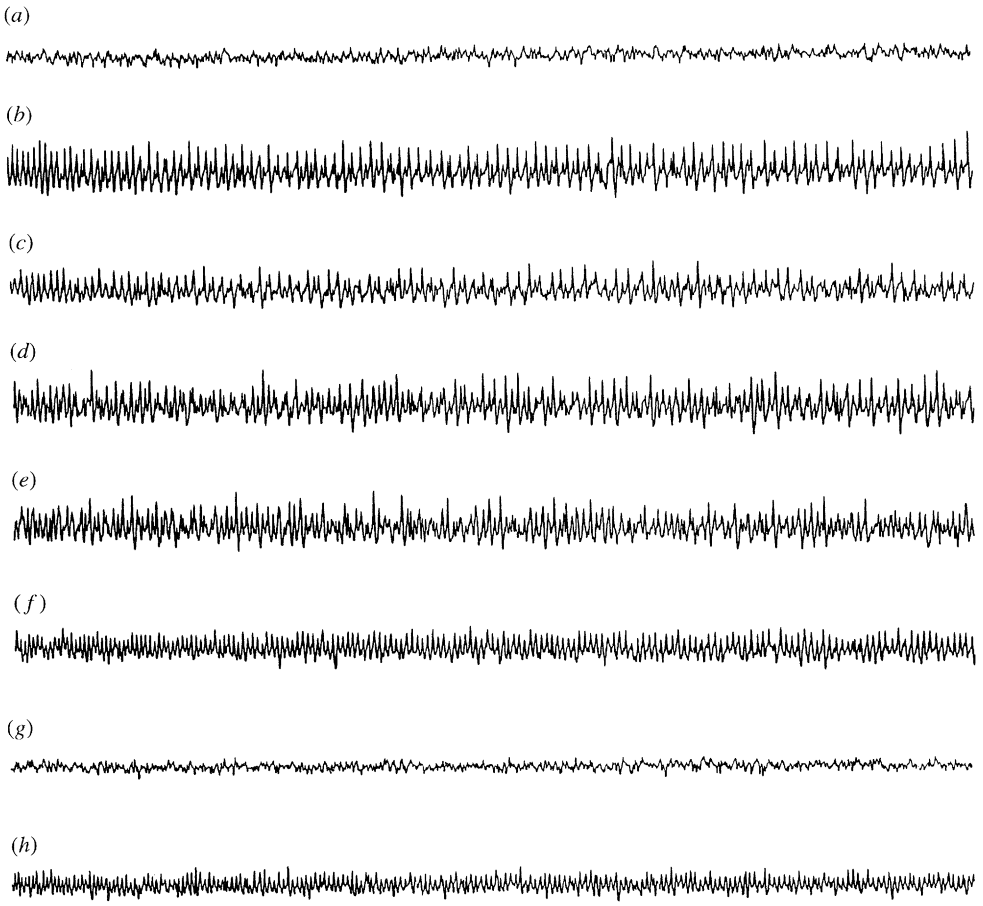

Figure 7. Representative responses from the lateral and the medial styloconic sensillia on the galea of *Trichoplusia ni* fifth instar larvae in response to: (*a*) 50 mM potassium chloride; (*b*) 10 mM sinigrin; (*c*) 10 mM sinigrin and 100 mM inositol; (*d*) 10 mM sinigrin and 60 mM sucrose; (*e*) 10 mM sinigrin, 100 mM inositol and 60 mM sucrose; (*f*) 60 mM sucrose, from lateral sensilla; (*g*) 50 mM potassium chloride; and (*h*) 10 mM sinigrin, from medial sensilla. All recordings were made from the same preparation. 50 mM potassium chloride served as the electrolyte.

plication, and an increase in spike amplitude with stimulus concentration. They demonstrated these parameters using extracts from an unacceptable non-host plant, *Canna generalis*. This deterrent compound stimulated a cell in the medial styloconic sensillium on the galea of *M. sexta*. In comparison, phagostimulants showed five- or tenfold shorter latency and rise in spike frequency and changes in spike amplitude did not appear to be directly related to concentration. van Loon (1990) also measured a long latency period prior to a tonic response in *P. brassicae* larvae responding to some anthocyanins.

In *M. configurata*, the MMInos cell (inositol-sensitive) adapted to 100 mM inositol within about 3 s, as did the MLSuc cell (sucrose-sensitive) to 60 mM sucrose. The phasic responses in both MMInos and MLSuc cells were of equal duration, about 2 s (figure 2). Ishikawa (1963) described an inositol-sensitive receptor in *Bombyx mori*. The adaptation curve given by Ishikawa for this cell in response to 100 mM inositol did not resemble that of the MMInos cell (figure 2), neither in its phasic nor tonic portion.

The *Mamestra* MLSuc cell possessed features that distinguished it from the *Trichoplusia* TLSuc cell, in that the former had a shorter phasic response (2 versus 5 s) and the tonic response in this cell terminated after about 3 s compared with more than 30 s for the TLSuc cell. Ishikawa (1963) described a sucrose-sensitive cell in *Bombyx mori*. It had a comparable adaptation curve to that of the MLSuc cell in response to 100 mM sucrose. Schoonhoven & Jermy (1977) described the adaptation of a sugar-sensitive cell in *P. brassicae* larvae. It had longer phasic and tonic phases than either MLSuc or TLSuc. The adaptation of a sugar-sensitive cell in the lateral styloconic sensillium of *Choristoneura fumiferana* larvae was more rapid than either MLSuc or TLSuc cells (Albert & Parisella 1988).

The adaptation profiles for the lateral sinigrin-sensitive cell in *M. configurata* (MLSin) and the lateral and medial sinigrin-sensitive cells in *T. ni* (TLSin and TMSin, respectively) studied here show generally similar features (figures 1, 4 and 5). Adaptation and dose–response characteristics (table 2 and elsewhere) for these cells fit the criteria for a deterrent type response, previously described by Schoonhoven & Blom (1988). The MLSin, TLSin and TMSin cells can, therefore, be classified as deterrent-sensitive cells, although more secondary plant compounds should be tested to substantiate this conclusion. These results are in agreement with an earlier behavioural investigation showing that this compound is a feeding deterrent (Shields & Mitchell 1995) for these species.

Table 2. *Selected response parameters for the lateral sinigrin-sensitive cell (MLSin) of* Mamestra configurata *and the lateral (TLSin) and medial (TMSin) sinigrin-sensitive cells of* Trichoplusia ni

	MLSin	TLSin	TMSin
threshold concentration/mM	0.16	0.02	0.06
sensitivity ratio compared to MLSin	1.0	8.0	2.7
dose-response range in log units	2	3	3
dose range used to calculate V_{max}/mM	0.16–30	0.02–5.0	0.06–10
maximum experimental response/(impulses s^{-1})	178	134	74
calculated V_{max}/(impulses s^{-1})	150	156	47
estimated dose at V_{max}/mM	$\geqslant$30	5	10
$S_{0.5}$/mM (concentration at half maximal response)	1.09	0.08	0.13
	MLSuc	TLSuc	TLSuc
	MLSin	TLSin	TMSin
duration of phasic phase (s)	2.1	5.1	5.1
	30.1	60.1	30.1
tonic response (impulses s^{-1})	34.5	81.1	81.1
	67.7	34.0	32.3

(*c*) *Comparative physiology of the sensory response to sinigrin*

Schoonhoven *et al.* (1992) grouped lepidopterous larvae from 15 species into those with only a medial deterrent neuron (six species), those with only a lateral deterrent neuron (one species), and those with both a medial and a lateral deterrent neuron (eight species). Thus, most species investigated (14 out of 15) have a deterrent neuron in the medial styloconic sensillum. Among crucifer-feeding insects, this medial cell type has been most thoroughly studied in *P. brassicae* (Ma 1972; Blom 1978*a*; van Loon 1990), where it has been found to respond to numerous secondary plant compounds, but not to glucosinolates (Blom 1978*a*; table 3). Sinigrin does not stimulate a cell in the medial styloconic sensillum of *M. configurata* (this paper), which does not mean that there is no deterrent cell in this sensillum. In *T. ni*, however, there is a medial cell that responds to sinigrin with approximately one-third the sensitivity of the very sensitive lateral deterrent cell in this species (table 3). It seems reasonable to conclude that the sinigrin-sensitive cell in the styloconic medial sensillum of *T. ni* is the classical deterrent cell so frequently found in lepidopterous larvae (Schoonhoven, *et al.* 1992). If this is true, it is to be expected that it will also respond to a wide range of secondary plant compounds.

Sinigrin, at concentrations 1 mM and above, effectively deters feeding in *M. configurata* and *T. ni*, especially when a minimally stimulating diet is used (Shields & Mitchell 1995). These behavioural data correlate well with the electrophysiological results reported here. We conclude that a high rate of firing from a cell in the lateral styloconic sensillum of *M. configurata* (MLSin) and the lateral and medial styloconic sensilla of *T. ni* (TLSin and TMSin) is at least partly responsible for mediating this feeding deterrence.

Host-plant ranges vary dramatically among the four species and are compared in table 3. *P. brassicae* is considered to be oligophagous, eating different orders of plants containing glucosinolates in the family Cruciferae (Verschaffelt 1910). *M. configurata* and *T. ni* are considered to be polyphagous, but differ considerably in their host-plant preferences (Shields & Mitchell 1995). *M. brassicae* is the most polyphagous of all. Its host plant range favours plants in at least 16 species from 10 families. It also eats leaves from at least 5 tree species from 4 families (Carter 1984).

Considering the lateral sinigrin-sensitive cells across all four species, an evolutionary or at least a host-plant relationship trend emerges. As one proceeds to species with tighter associations with the Cruciferae, the sensitivity of this cell to sinigrin (and by extension, other glucosinolates) becomes progressively less (table 3). At the extreme, the lateral deterrent cell of *P. brassicae* is insensitive to sinigrin. This trend may reveal a process that occurs in evolutionary time as a species becomes even more closely associated with a plant family. In the case of *P. brassicae*, for example, it is suggested that the lateral deterrent cell has lost its sensitivity to sinigrin as a necessary consequence of crucifer specialization (Schoonhoven 1991).

Table 3. *Evolutionary host-plant relations and varying characteristics of the lateral and medial sinigrin-sensitive cells in four crucifer-feeding insects*

		lateral sensillum	lateral sensillum	medial sensillum
species	host habit	sensitivity of deterrent cell to sinigrin	sensitivity of specialized cell to sinigrin	sensitivity of deterrent cell to sinigrin
Pieris brassicae	crucifer specialist	insensitive	very high (10^{-4} mM)[a]	insensitive
Mamestra brassicae	extremely polyphagous	low (1 mM)[a]	no response	very low (5 mM)[a]
Mamestra configurata	polyphagous, associated with crucifers	medium (0.16 mM)	no response	no response
Trichoplusia ni	extremely polyphagous	high (0.02 mM)	no response	high (0.06 mM)

[a] Indicates reference from Blom (1978*a*).

M. brassicae is very polyphagous and yet has a moderately low sensitivity to sinigrin (both lateral and medial deterrent cells), where one might expect it to show high sensitivity (similar to *T. ni*). This apparent paradox can be overcome if one considers the processes leading to developing an association with crucifers and to the maintenance of polyphagy, separately. There is no *a priori* reason for an insect to leave old multiple hosts behind to adapt somewhat better to a particular plant family. As its name implies, *M. brassicae*, although highly polyphagous, is also well adapted to crucifers. Thus, its lateral and medial deterrent cells are relatively insensitive to sinigrin, suggesting an evolutionary desensitization similar to that seen in *P. brassicae*, although not as complete. Nevertheless, the sensory features that allow it to be polyphagous remain intact.

M. configurata, on the other hand, is also adapted to crucifers and not notoriously polyphagous. Its medium sensitivity to sinigrin, via the lateral deterrent cell, is greater than one might expect. Its medial deterrent cell (assuming there is one present, as in most lepidopterous larvae), however, is insensitive to sinigrin, presumably due to the same desensitization process discussed above. This species appears to have lost some ability to deal with a wide range of hosts (compared with *M. brassicae*) and yet is moderately well adapted to crucifers (as measured by deterrent cell sensitivity).

P. brassicae, the crucifer specialist, is sensitive to sinigrin via a cell in the lateral sensillum and to sinalbin, an aromatic glucosinolate, in the medial sensillum. These cells are more or less glucosinolate specialists (Verschaffelt 1910; Schoonhoven 1967; Ma 1972) and do not respond to a wide range of secondary plant compounds. Another cell in the lateral sensillum has been shown to be sensitive to potentially deterrent compounds (van Loon 1990). This phenolic-sensitive cell may be the *P. brassicae* analogue to the medial sinigrin-sensitive (deterrent) cell in the three other species. In *P. brassicae*, adaptation to crucifers seems to have involved: (i) desensitization of the deterrent cell to sinigrin, as argued above; and (ii) development of more specific glucosinolate sensitivity in another cell (also in the lateral sensillum), which presumably mediates host recognition and, therefore, signals acceptance.

Additional support for this entire scenario would be provided if it could be demonstrated that the medial deterrent cell (sinigrin-sensitive) of *M. configurata* and *T. ni* was also sensitive to a wide range of potential feeding deterrents. Such data are currently not available.

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