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Taste detection of phytoecdysteroids in larvae of *Bombyx mori*, *Spodoptera littoralis* and *Ostrinia nubilalis*

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Abstract

A number of plants produce significant amounts of phytoecdysteroids that can disrupt the hormonal levels of insects feeding upon them. Insects equipped with taste receptors sensitive to phytoecdysteroids are able to avoid such plants. How common is this strategy? By recording from the lateral and medial sensilla styloconica in two polyphagous species (*Ostrinia nubilalis* and *Spodoptera littoralis*) and in a monophagous species (*Bombyx mori*), we tested whether the receptors could detect three commonly occurring phytoecdysteroids 20-hydroxyecdysone (20E), ponasterone A (ponA) and ecdysone (E). In *B. mori*, 20E and ponA elicited dose-dependent responses with a threshold of 1 μ M only in the medial sensilla. In *O. nubilalis*, 20E, E and ponA elicited responses at threshold of 1 μ M in both sensilla. In *S. littoralis*, 20E elicited responses with a threshold of 10 μ M in both sensilla. By means of behavioural choice tests, we show that 20E is an effective feeding deterrent for *O. nubilalis* and *S. littoralis* first instar larvae. This suggests that the perception of phytoecdysteroids is more common among phytophagous lepidoptera than previously thought, although their toxicity or antifeedancy varies between species. © 2002 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Phytoecdysteroids are steroidal compounds produced by plants that interfere with insect ecdysis (Lafont, 1997). About 5–6% of plants species, including primitive groups such as ferns, contain measurable amounts of phytoecdysteroids (Dinan, 1998). Their concentrations vary over a wide range (up to 1 mM: Blackford and Dinan, 1997b; Dinan et al., 1999). The most common phytoecdysteroids are 20-hydroxyecdysone (20E), polypodine B, makisterone A and ponasterone A (ponA) (Lafont et al., 1991). They are generally considered as secondary compounds that protect plants against attack by phytophagous insects, either by disturbing development or reducing food intake (Bergamasco and Horn, 1985; Camps, 1991; Lafont, 1997; Adler and Grebenok, 1999). Support for their role as defensive compounds also comes from evidence showing that phytoecdys-

teroid accumulation is enhanced after injury and insect attack (Schmelz et al., 1998; Schmelz et al., 1999). Ecdysteroids could be useful for improving plant resistance to insect pests because they are innocuous to vertebrates (Sláma and Lafont, 1995). Although their toxic effect on herbivorous insects is moderate as compared to modern insecticides, they deter feeding in several herbivorous insects suggesting that they could be used within the framework of a ‘push–pull’ strategy (Khan et al., 2000).

Lepidopteran larvae are especially interesting for studying how ecdysteroids can deter feeding because they possess few taste sensilla, grouped on three organs on the cephalic capsule. These are the galea with two taste styloconic sensilla, the maxillary palps with five small-sized peg-shaped taste sensilla, and the epipharyngeal organ (Bernays and Chapman, 1994). Each individual sensillum houses four taste neurones with different specificities (except the epipharyngeal which house three). Galeal sensilla are the most easily accessible to electrophysiological investigations. Two types of neurones are involved in the detection of plant secondary metabolites: token stimulus receptor neurones, which

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stimulate feeding, and deterrent receptor neurones that signal inhibition of feeding (Schoonhoven, 1987). While the first are considered as specialised, the second respond to a broad array of compounds. Earlier observations showed the presence of a taste neurone sensitive to phytoecdysteroids that mediated deterrence in insects that are specialised feeders of one plant family like the oligophagous *Pieris brassicae* on cruciferous plants (Ma, 1972) and the monophagous *Bombyx mori* on *Morus* sp. (Tanaka et al., 1994b). We have also recently found such a cell responding to 20E and to ponA in the polyphagous *Mamestra brassicae* (Descoins and Marion-Poll, 1999).

Given the broad distribution of phytoecdysteroids in plants, polyphagous species are more likely to encounter such compounds than specialist insects. Are such insects able to detect these compounds as sensitively as specialist insects? We evaluated the sensory detection of three phytoecdysteroid molecules (20E, E and ponA; Fig. 1) by larvae of two polyphagous species, *Ostrinia nubilalis* and *Spodoptera littoralis*. We further submitted larvae of these species to a behavioural feeding choice test in order to evaluate if 20E was a deterrent compound to them. This was done on L1 larvae, which is a critical stage for plant colonisation. We also used *B. mori*, which possesses a deterrent taste neurone responding to 20E (Tanaka et al., 1994b). Its sensitivity towards E and ponA has not yet been described. Interestingly, while L5 larvae actively avoid 20E in their diet, L3 larvae seem to lack this behavioural response (Tanaka et al., 1994b). We thus checked if the sensitivity to 20E was maintained across larval stages by comparing electrophysiological responses in L3 and L5 larvae. The sensitivity to 20E and other compounds tested in this study does not seem

to change across larval stages. Our observations suggest that phytoecdysteroids are detected by several polyphagous species and that, when given a choice, these insects avoid eating diets containing these compounds.

2. Materials and methods

2.1. Biological material

B. mori larvae originating from INRA Unité Nationale Séricicole (La Mulatière) were reared on fresh mulberry leaves. *O. nubilalis* and *S. littoralis* larvae, respectively, originating from INRA Le Magnereau and INRA La Minière were reared on an artificial diet (Poitout and Bues, 1970). These larvae were maintained in transparent plastic boxes at 20 °C under a 16 h light/8 h dark photoperiod and 70% relative humidity. Recordings were performed on 24 h starved larvae, which had recently moulted (*B. mori*: L3 and L5 larvae; *O. nubilalis*: L5 larvae; *S. littoralis*: L3 larvae).

2.2. Chemicals

20-Hydroxyecdysone (20E; 95% min. purity), potassium chloride (KCl), fructose, leucine and proline (TLC grade, 98% purity) were purchased from SIGMA-ALDRICH. Ecdysone (E; HPLC: 98% or more purity) and (ponA; HPLC: ≥ 98 purity) were gifts from R. Lafont (Ecole Normale Supérieure). All compounds were dissolved in ultra pure water with 10^{-2} M KCl, which served both as a control and as a conductive solution. Ecdysteroids were used at concentrations ranging from

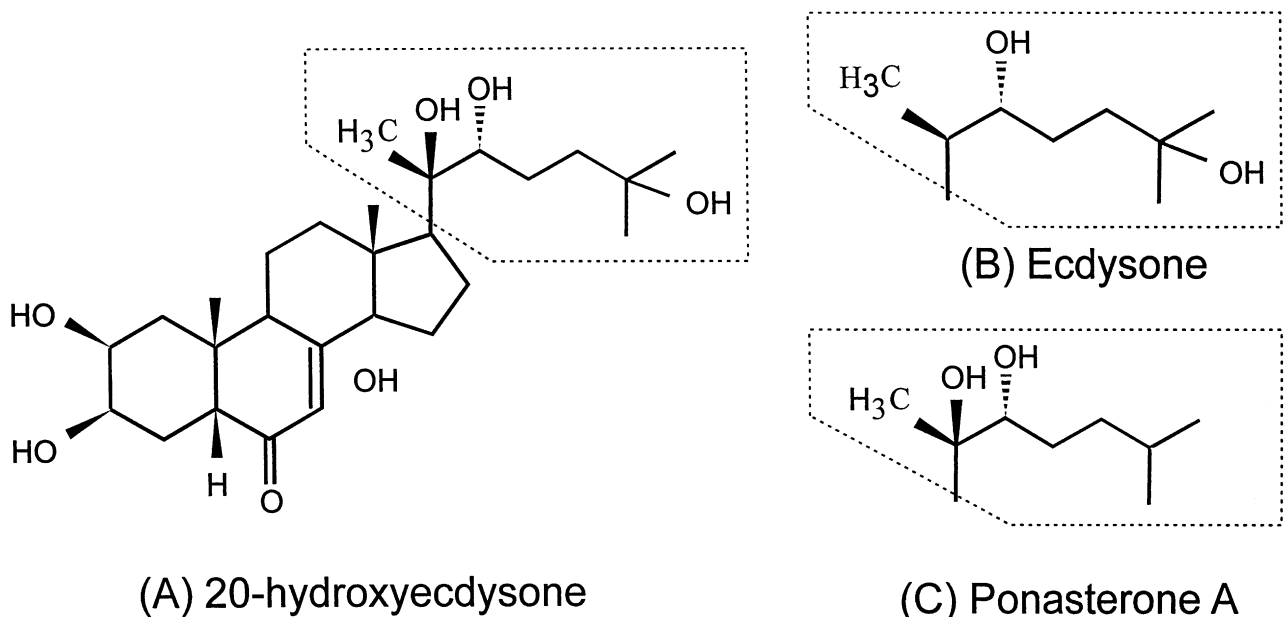


Fig. 1. Structures of the phytoecdysteroids evaluated in this study. (A) Structure of the 20E molecule; the lateral chain in the other molecules is encircled by a dotted line. (B) Structure of the lateral chain of ecdysone (E). (C) Structure of the lateral chain of ponA.

10^{-8} to 10^{-3} M. PonA was dissolved in 5% ethanol, due to its relative insolubility in water. Pilot tests confirmed that 5% ethanol solutions had a negligible impact on the taste sensilla. All solutions were kept at 4 °C for less than one month.

2.3. Electrophysiological recordings

Recordings were performed on the lateral and medial sensilla, located on the galea. An earthed silver electrode (0.4 mm diameter) was introduced into the cephalic cavity through the severed abdomen. Stimulus solutions were brought in contact with the tip of the sensilla under visual control (Wild M10, Leica) using a glass electrode (tip diameter about 20 μ m) filled just before recording. Electrophysiological activity was recorded using a TastePROBE amplifier (Marion-Poll and Van der Pers, 1996) and further amplified and filtered (CyberAmp 320, Axon Instruments; gain: 1000–2000; 8-poles Bessel band-pass filter: DC/0.1–4000 Hz). Data were recorded and stored on a computer with a data acquisition card (DT 2821, Data Translation, 12 bits precision, sampling rate: 10 kHz) driven under ATLSPK (Marion-Poll and Tobin, 1991). Each recording lasted 2.5 s and was triggered by a pulse delivered by the amplifier on the initial contact of the electrode with the sensillum.

Each set of recordings was performed by stimulating the medial and lateral maxillary sensilla of one side. Each stimulus was delivered twice, at intervals ranging between 30 and 50 s, keeping 3–4 min between stimuli with different compounds or at different concentrations. The protocol started with the presentation of control stimuli: KCl (10^{-2} M), a sugar (10^{-2} M fructose), and two amino acids (leucine and proline at 10^{-2} M). These stimuli were meant to evaluate the responsiveness to feeding stimulants, and thus to elicit responses from neurones presumably not stimulated by phytoecdysteroids. The two amino acids, leucine and proline, were chosen because of their availability in plants and their suggested role in host plant selection (Derridj et al., 1996). Following the presentation of the control stimuli, increasing concentrations from one test compound were presented twice (20E and E at ten-fold dilutions between 10^{-8} and 10^{-3} M; ponA: 10^{-8} , 10^{-6} , 10^{-4} M).

2.4. Data analysis

The total number of recordings analysed during this study was 850 for *B. mori* ($n = 30$ larvae, two larval stages), 1030 for *O. nubilalis* (15 larvae), and 910 for *S. littoralis* (15 larvae). Data files were analysed with AWAVE (Marion-Poll, 1995; Marion-Poll, 1996), to which new analysis modules were added. Spikes were first detected from the signal, filtered by a running median algorithm spanning a 6 ms window (Fiore et al., 1996). This filter efficiently compensates for baseline

shifts and yields better detection conditions than the low-pass filtered derivative used in previous studies (Marion-Poll and Tobin, 1991). Each spike was described by its occurrence time and a 60-point array (6 ms), centred on the maximum reached after the first threshold crossing. These arrays were then subjected to various measurements (see later) to separate them into different amplitude or waveform classes.

The magnitudes of the responses were evaluated by counting the total number of spikes during the first second of the recordings. These responses were subjected to an ANOVA analysis using SAS (release 6.12 for X Windows; SAS Institute Inc., NC, USA). The model considered interactions between the following factors: chemicals, concentration, sensillum type and larval stage. A Bonferroni (Dunn) *t*-test was used to split responses into groups of significance and to compare them to the responses to 10^{-2} M KCl.

The time course of the responses over the duration of the recordings was described with post-stimulus histograms (PSTHs), using bins of 50 ms. The regularity of the firing rate was evaluated with interspike interval histograms (ISIHS) and autocorrelograms, using bins of 1 ms. ISIHS and autocorrelograms were computed from 0.5 to 2.5 s. These histograms were intended to measure the regularity of the responses during the tonic phase of the responses. All histograms were normalised in units of spk s^{-1} , according to Abeles (1985).

2.5. Behavioural observations

In order to check if 20E had an effect on the feeding behaviour, groups of 25 L1 larvae of *O. nubilalis* and 30 *S. littoralis*, were offered a choice between two substrates. One substrate received 26 μ l of 3×10^{-3} M 20E in acetone while the other received 26 μ l of solvent alone. A control test was run in parallel, using disks with only acetone. A no-choice test was performed with two other groups of larvae, which were offered a single disk, that was either treated or not. The disks (1 cm diameter and 3–4 mm height) were cut out of a layer of food medium freshly prepared and placed on the bottom of a 5 cm diameter Petri dish. Freshly hatched larvae were deprived of food during 24 h, and then delicately transferred to the centre of the Petri dish. The number of larvae found on each disk or within a radius less than 0.5 cm from its circumference was counted after 1, 2, 3, 5 and 24 h. These tests were repeated 10 times. Feeding choice experiments were analysed using paired Student's *t*-tests for the choice tests and unpaired *t*-test for the no-choice experiments using SAS/ASSIST interactive procedures.

3. Results

3.1. Overall firing response

In *B. mori*, *O. nubilalis* and *S. littoralis*, extracellularly recorded spikes ranged between 0.3–2 mV, 0.3–3 mV and 0.1–2 mV for the three species, respectively, superimposed on a 100–200 μ V (peak-to-peak) noise. Spikes were discriminated from background with an adjustable threshold set across the signal, compensated for baseline drifts using a digital filter. This procedure thus extracted only the most conspicuous spikes. In some recordings, spikes of 200–300 μ V were visible but were too close to the noise to be taken into account.

Responses to 10^{-2} M KCl were 7.7 ± 1.2 (average \pm s.e.m.) versus 15.2 ± 1.8 spk s^{-1} on the lateral and medial sensilla of *B. mori*, 6.0 ± 0.7 vs. 10.9 ± 1.3 spk s^{-1} in *O. nubilalis*, and 31.2 ± 1.7 vs. 33.4 ± 1.9 spk s^{-1} in *S. littoralis* (Fig. 2). These values represent the background activity since 10^{-2} M KCl was used as a solvent for all other stimuli.

In *B. mori*, the medial sensilla responded prominently to 10^{-3} to 10^{-5} M 20E and 10^{-4} M ponA (Fig. 2A; $P < 0.05$). In the lateral sensilla, only leucine elicited a response significantly higher than the control ($P < 0.05$). There was a significant effect of concentration for the responses of the medial sensilla to 20E ($F = 29.9$, $df = 5$, $P = 0.0001$) and PonA ($F = 14.4$, $df = 5$, $P = 0.0001$), but only marginal for E ($F = 2.25$, $df = 5$, $P = 0.04$). Responses of the medial and lateral sensilla to 20E and the control solutions were recorded in L3 ($n = 5$) and L5 larvae ($n = 6$) (medial sensilla: Fig. 3). There was no statistical difference between the two groups of observations, either in the lateral ($F = 0.00$, $df = 1$, $P = 0.98$) or in the medial sensilla ($F = 0.18$, $df = 1$, $P = 0.67$).

In *O. nubilalis* (Fig. 2B), both the medial and the lateral sensilla gave dose-related responses to 20E and E (medial: $F = 23.5$ and 33.06 , $df = 5$, $P = 0.001$; lateral: $F = 20.1$ and 53.5) and to ponA (medial: $F = 4.1$, $df = 2$, $P = 0.02$; lateral: $F = 5.7$, $df = 2$, $P = 0.008$). For the lateral sensilla, leucine was also quite active (42 spk s^{-1} ; Bonferroni $P < 0.05$), whereas fructose was most effective on the medial sensilla (76.6 spk s^{-1} ; Bonferroni $P < 0.05$).

In *S. littoralis*, 20E elicited a dose-dependent response (lateral: $F = 11.51$, $df = 5$, $P = 0.0001$; medial: $F = 13.55$, $df = 5$, $P = 0.0001$), ranging from 25 to 60 spk s^{-1} in both the lateral and medial sensilla (Fig. 2C). Neurons in the lateral sensillum were quite sensitive to fructose (86 spk s^{-1} ; Bonferroni $P < 0.05$), leucine (76 spk s^{-1} ; $P < 0.05$) and proline (57 spk s^{-1} ; $P < 0.05$), whereas in the medial sensilla, leucine elicited a smaller response than KCl (19.7 spk s^{-1} ; $P < 0.05$).

3.2. Time course and spike train regularity of the responses

In *B. mori*, the responses to 20E at 10^{-3} to 10^{-5} M exceeded the responses to 10^{-2} M KCl in the medial sensilla, during the whole time-course of stimulation (Fig. 5A). In response to 10^{-6} M 20E, the firing activity was different from KCl after a 500 ms delay. The maximum firing activity was reached 50, 100 and 200 ms after the beginning of the stimulation with 20E at 10^{-3} , 10^{-4} and 10^{-5} M, respectively. The responses were mainly monocellular (Fig. 4A).

In *O. nubilalis*, both the medial and the lateral sensilla responded to 20E, E, and ponA. The medial sensilla were the most responsive (Fig. 5B and C). The smallest dose that elicited responses to 20E was 10^{-6} M. At higher doses, the maximum firing rate was reached 150–300 ms after the initial contact. The responses were then maintained tonically. Responses to E followed a similar pattern, with a detection threshold between 10^{-6} and 10^{-5} M. The maximum firing rate was reached about 350 ms after the initial contact. With ponA, responses were visible at 10^{-4} M and 10^{-6} M. The responses elicited by 20E was mainly monocellular (Fig. 4B and C); responses to E were often involving two cells.

In *S. littoralis*, only 20E elicited a response in both lateral and medial sensilla. The time course of the response was also phasi-tonic (Fig. 5D and E). The responses elicited by 20E involved in most cases two or three cells. In contrast to what was found in *B. mori* and *O. nubilalis*, the latencies of the responses to 10^{-6} M 20E were short.

3.3. Behavioural responses

First larval instar of *O. nubilalis* and *S. littoralis*, when placed in a Petri dish, spent more time in close vicinity to an acceptable food disk (within a 1 cm radius of its circumference) rather than wandering elsewhere. We recorded the number of larvae close to food disks at regular time intervals (Table 1). When given a choice between two food disks, the larvae were distributed evenly around the food disks ((B) in Table 1), unless one disk was treated with 3×10^{-3} M 20E ((A) in Table 1 and Fig. 6). In a non-choice situation, *O. nubilalis* larvae were found significantly less in the vicinity of 20E treated disks as compared to control disks ((C) in Table 1, right column), while significantly more *S. littoralis* larvae were found close to the treated disk ((C) in Table 1, left column).

4. Discussion

In this work, we have compared the electrophysiological responses of taste receptors from three species of

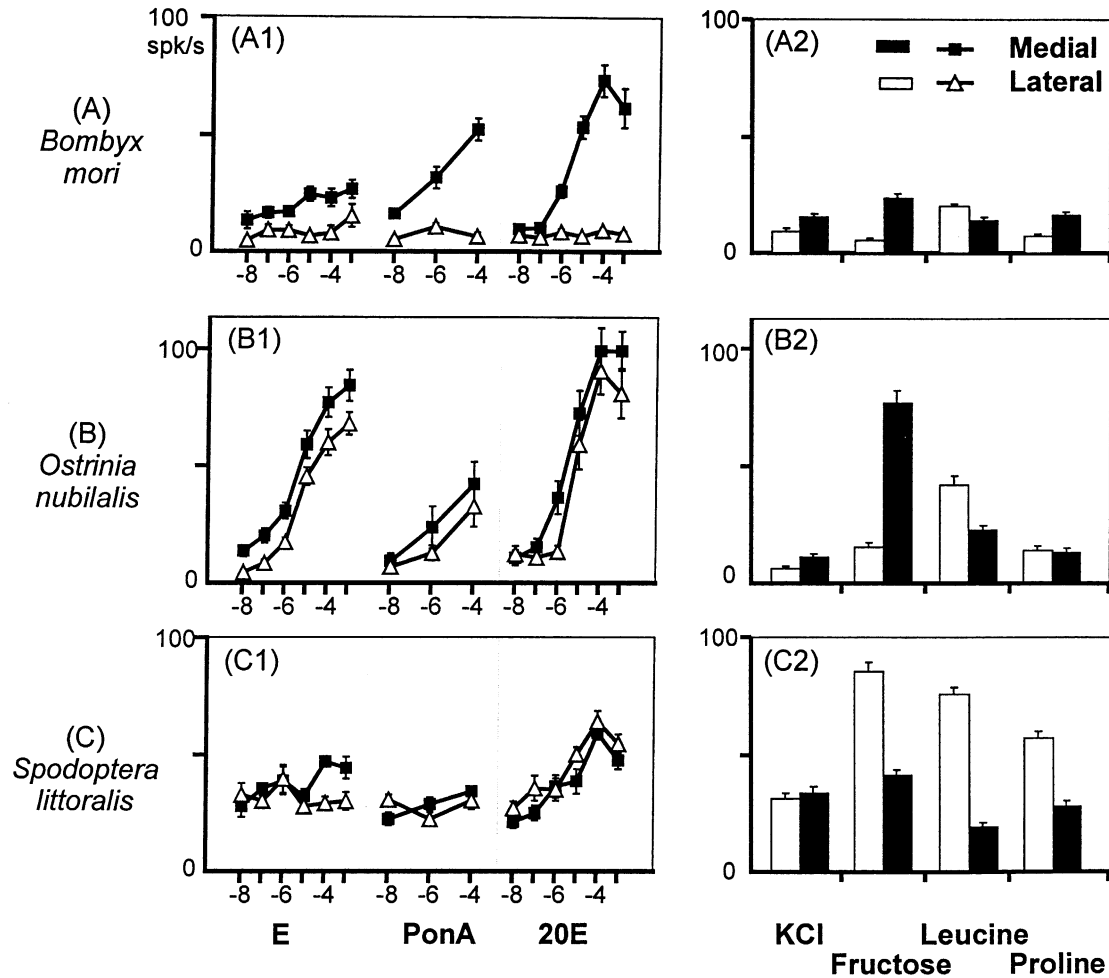


Fig. 2. Sensory responses of the lateral and medial sensilla styloconica of the three insect species. (A) *B. mori*: combined data from the third and fifth instars. (B) Responses from *O. nubilalis* larvae: fifth instar. (C) *S. littoralis*: third instar. On the left (A1, B1, C1), responses to ascending concentrations of E, ponA and 20E. Ordinates: number of spikes per second (spk s^{-1}). Abscissa: compounds evaluated. Each data point represents the average number of action potentials during the first second of the recordings (mean \pm standard error of the mean; $n = 10$ stimulations). Black squares: responses from the medial sensilla styloconica; open triangles: responses from the lateral sensilla styloconica. On the right (A2, B2, C2), responses to a sugar (fructose), a salt (KCl) and two amino acids (leucine and proline). The sugar and the amino acids were dissolved in a solution of 10^{-2} M KCl to reach a 10^{-2} M concentration. Black bars: responses from the medial sensilla; empty bars: responses from the lateral sensilla. Each bar represents the mean \pm s.e.m. from 40 to 50 stimulations.

moths, *B. mori*, *S. littoralis* and *O. nubilalis*. The larvae of these insects have similar sensory organs. We have recorded the electrical activity from two prominent taste sensilla located on the galea. These sensilla are known to house taste neurones, which are likely to be involved in the perception of plant secondary compounds such as phytoecdysteroids.

In *B. mori*, we have found a monocellular response to 20E and to ponA but not to E in the medial sensilla styloconica. This matches previous observations from Tanaka et al. (1994b) who reported that 20E activates a 'bitter receptor' in the medial sensilla styloconica.

In *S. littoralis*, we obtained multicellular dose-related responses to 20E but not to ponA in both lateral and medial sensilla. We obtained vigorous responses with fructose, leucine and proline in the lateral sensilla, and with fructose in the medial sensilla.

In *O. nubilalis*, we observed responses to 20E, ponA and E in both lateral and medial sensilla styloconica. Differences were observed in the intensity of the responses between the lateral and medial sensilla, the intensity of the responses being larger in the medial. This is the first time that responses to such deterrent compounds have been demonstrated in this polyphagous insect species.

4.1. Is perception of phytoecdysteroids mediated by a deterrent cell?

Measuring the responsiveness of taste receptors does not give any cue about the effect of phytoecdysteroids on behaviour, except if one could find parameters that are specific to the activation of 'deterrent' receptors. A few parameters were proposed by Peterson et al. (1993),

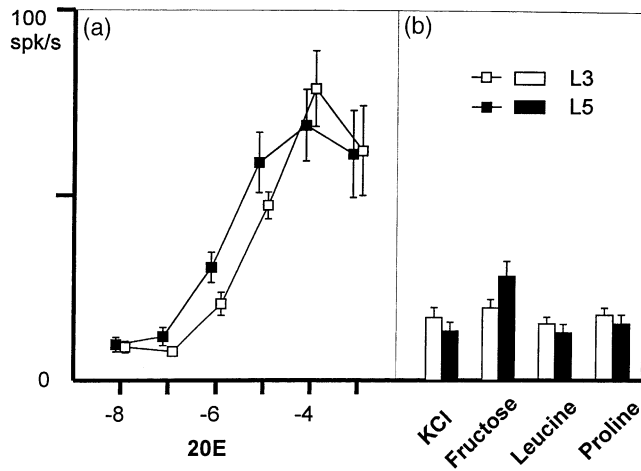


Fig. 3. Comparison of the sensory responses of the medial sensilla styloconica of *B. mori* at the third and fifth instars. (a) Responses to 20E at increasing concentrations. Each point of the dose–response curve represents the mean \pm sem of 10–12 stimulations. (b) Responses to KCl, fructose, leucine and proline at 10^{-2} M. Each bar represents the mean \pm sem of 20 to 26 stimulations. Abscissa and ordinates: same as Fig. 2. Black squares and bars: fifth instars, white squares and bars: third instars.

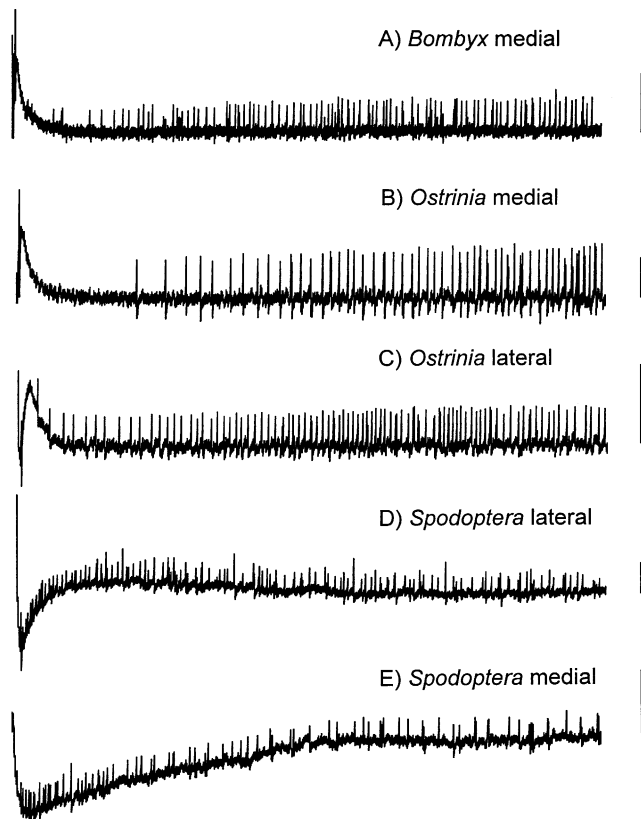


Fig. 4. Recording samples of responses to 10^{-6} M 20E in (A) *B. mori* medial sensilla. (B)–(C) *O. nubilalis* medial and lateral sensilla respectively. (D)–(E) *S. littoralis* lateral and medial sensilla respectively. Right vertical bar: 1 mV; trace duration: 2.5 s.

when analysing responses from *Manduca sexta* larvae to chemical extracts from a non-host plant *Canna generalis*. The authors stated that the fractions from *C. generalis* extracts which inhibited feeding were activating a specific taste cell called a ‘deterrent cell’, located in the medial sensilla styloconica. This taste cell responded with unique temporal response parameters (long latency and slow rise of spike frequency at the onset) and a characteristic increase of spike amplitude at the beginning of stimulation. More recent papers described the activation of a deterrent cell to other bitter compounds in *M. sexta* or *Pieris* larvae in similar terms (Glendinning and Hills, 1997; van Loon and Schoonhoven, 1999).

We have analysed responses observed in the medial sensilla of *B. mori* to 20E and ponA, and in the lateral and medial sensilla of *O. nubilalis* to 20E, ponA and to a lesser extent to E. They exhibit characteristics of long latency, slow rise of firing frequency and spike amplitude increase during the initial response which are consistent with the parameters proposed above. Thus, according to these criteria, the responses recorded in *B. mori* (in the medial cell: 20E and ponA) and *O. nubilalis* (in both cells: 20E, ponA and E) could originate from the activation of deterrent cells.

Apart from the higher sensitivity of these cells to 20E (threshold 10^{-6} M or below) as compared to sugars or salts (threshold 10^{-3} M), the temporal characteristics of the responses were also different. The responses to sugars, salts and amino acids were phasic, with an initial burst of spikes followed by a lower firing rate. The responses to 20E were tonic and regular, even when the response onset was delayed by 100–200 ms, as seen for lower dosages (less or equal to 10^{-6} M). These observations suggest that only one neurone is active in *B. mori* and *O. nubilalis* in response to 20E and ponA. In contrast to this, the responses to E observed in *O. nubilalis* involved more than one neurone; this was also the case for the responses to 20E observed in *S. littoralis*. These observations challenge the ‘labelled line’ coding hypothesis, which is implicit when describing one neurone as a ‘deterrent cell’. Further studies are needed to resolve if the activity of both neurones is needed to induce anti-feedancy, which would imply a combinatorial coding (Schoonhoven et al., 1992).

4.2. Deterrent effect of phytoecdysteroids

The role of phytoecdysteroids as feeding deterrents requires confirmation from behavioural observations. On *B. mori* this information was already available. Tanaka et al. (1994b) observed that 20E but not E was deterrent to L5 larvae. In this species, the sensitivity to phytoecdysteroids of one cell within the medial sensilla matches the contents in its host plant *Morus* sp. Leaves from this plant contain very low levels of 20E (1–2 ppm)—under the threshold sensitivity—and no detectable levels of E

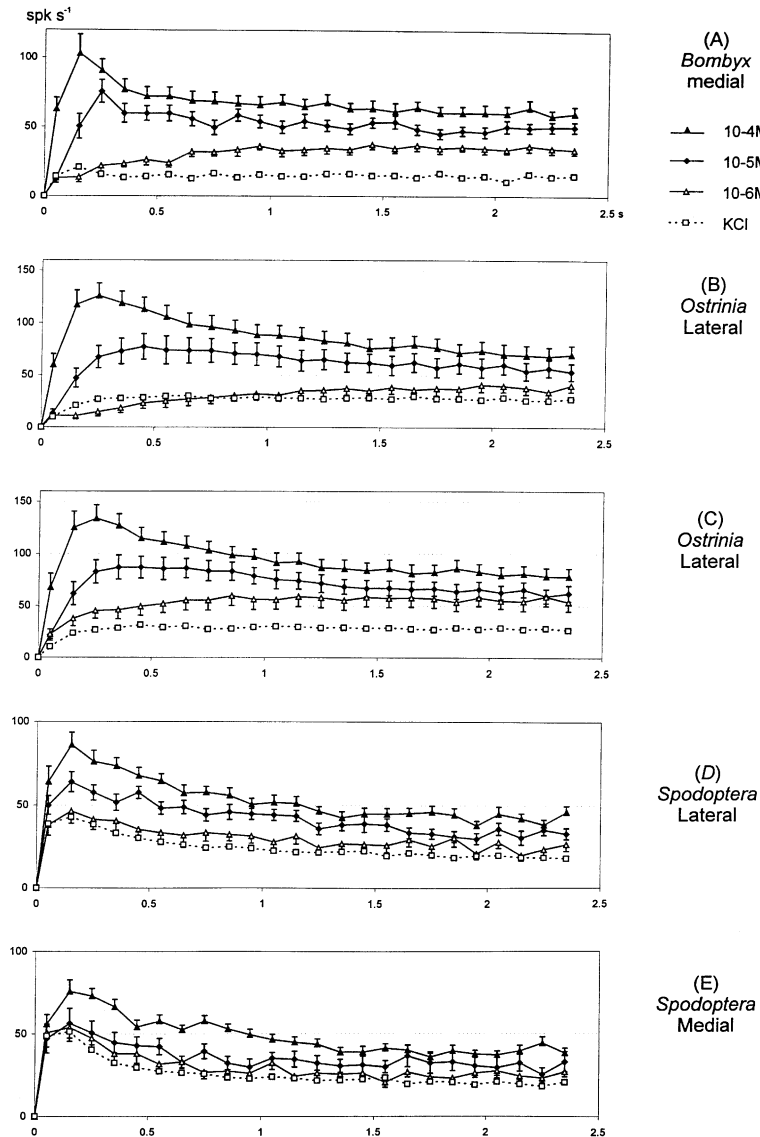


Fig. 5. Time course of the sensory responses to serial dilutions of 20E and to 10^{-2} KCl. Each curve represents averaged data from 10 trials (two trials on one sensillum from each of five animals). Individual data points are the average instantaneous frequency of the neurones, measured during consecutive 500 ms bins, over the total 2.5 stimulus period. Ordinates: action potentials/s, Abscissa: time course of the stimulation in s. (A) *B. mori* medial sensilla. (B)–(C) *O. nubilalis* medial and lateral sensilla respectively. (D)–(E) *S. littoralis* lateral and medial sensilla respectively.

(Blackford and Dinan, 1997b). In *S. littoralis*, the situation is less clear. Earlier behavioural observations indicated that *S. littoralis* larval feeding is not reduced by 20E nor by other phytoecdysteroids. Our current observations suggest that when given a choice, a significant avoidance to phytoecdysteroids is observed. In *O. nubilalis*, we have shown that 20E is a strong antifeedant and that both lateral and medial sensilla house a cell responding to very low levels of phytoecdysteroids. Thus, the behavioural effects of phytoecdysteroids on these three species of larvae matches the electrophysiological observations, i.e. a behavioural effect is correlated to the activation of at least one taste cell in the medial and/or the lateral sensilla styloconica of the galea.

Although there is apparently a good match between

the electrophysiological and the behavioural observations, this might not always be true. On *B. mori*, earlier experiments suggested that 20E had almost no effect on the feeding of L2 to L3 instars while it was quite efficient on L4 and L5 instars (Tanaka et al., 1994b). From these results, one could infer that the perception of 20E vary between stages, i.e. that the intensity of the response to 20E should be lower in taste sensilla from L3 larvae as compared to L5 larvae. Our results demonstrate that gustatory responses to 20E and ponA of the medial sensilla are comparable in L3 and L5 larvae. These conflicting observations would need a more specific testing, for example using insects from the same strain as in the earlier study.

Table 1

Behavioural responses of first instar larvae of *S. littoralis* (10 trials with $n = 30$ insects) and *O. nubilalis* (10 trials with $n = 25$ insects) (A) given a choice between two food disks treated with 3×10^{-3} M 20E (20E) or with solvent alone (control), (B) between disks treated with solvent only (control vs. control) or (C) given no choice to feed on a food disk treated with 3×10^{-3} M 20E (20E) or with solvent alone (control). Numbers reported indicate the mean number of insects found in close vicinity to the food disk (see Section 2) \pm s.e.m. Stars on the left column indicate the level of significance for the hypothesis that means are different between treatments (NS = nonsignificant; * $P > 0.1$; ** $P > 0.01$; *** $P > 0.001$)

	<i>S. littoralis</i>			<i>O. nubilalis</i>		
Choice (A)	Control	20E		Control	20E	
1 h	11.0 \pm 0.6	5.2 \pm 0.5	***	5.7 \pm 0.6	4.1 \pm 0.9	NS
2 h	14.8 \pm 0.5	6.2 \pm 0.9	***	12.0 \pm 0.6	3.9 \pm 0.8	***
3 h	14.0 \pm 0.8	7.5 \pm 1.1	***	17.4 \pm 0.8	4.1 \pm 1.0	***
5 h	15.9 \pm 1.0	9.7 \pm 1.4	*	16.8 \pm 0.8	1.9 \pm 0.6	***
24 h	11.6 \pm 1.7	9.6 \pm 1.2	NS	14.5 \pm 0.6	5.1 \pm 0.6	***
(B)	Control	Control		Control	Control	
1 h	8.9 \pm 0.6	6.3 \pm 0.6	*	4.8 \pm 0.6	5.5 \pm 0.5	NS
2 h	10.1 \pm 0.7	8.2 \pm 0.5	NS	7.8 \pm 0.8	9.2 \pm 0.8	NS
3 h	7.6 \pm 0.8	6.7 \pm 0.5	NS	11.1 \pm 0.7	10.2 \pm 0.7	NS
5 h	9.8 \pm 1.2	8.6 \pm 0.7	NS	10.7 \pm 0.7	10.2 \pm 0.7	NS
24 h	7.9 \pm 0.5	12.7 \pm 1.8	*	9.6 \pm 0.7	9.9 \pm 0.6	NS
Non-choice (C)	Control	20E		Control	20E	
1 h	13.6 \pm 1.0	18.0 \pm 1.0	**	5.7 \pm 0.6	4.1 \pm 0.9	NS
2 h	18.2 \pm 0.6	14.3 \pm 2.3	NS	12.0 \pm 0.6	3.9 \pm 0.8	***
3 h	17.2 \pm 0.7	16.0 \pm 2.6	NS	17.4 \pm 0.8	4.1 \pm 1.0	***
5 h	20.0 \pm 1.2	15.3 \pm 2.0	*	16.8 \pm 0.8	1.9 \pm 0.6	***
24 h	16.7 \pm 1.5	25.2 \pm 1.1	***	14.5 \pm 0.6	5.1 \pm 0.6	***

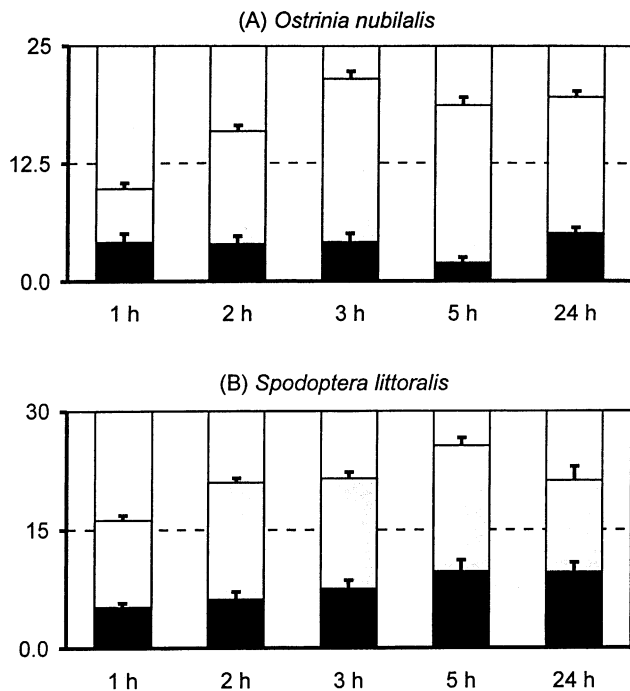


Fig. 6. Behavioural responses of first instar larvae to a choice between two food disks, treated with 20E (26 μ l of 3×10^{-3} M in acetone) or with the solvent alone. (A) *O. nubilalis* larvae (10 trials with 25 larvae each). (B) *S. littoralis* larvae (10 trials with 30 larvae each). Bars indicate the mean number of larvae (mean \pm sem) found in the vicinity of a food disk treated with 20E (black bars), solvent alone (grey bars) or wandering outside these areas (white bars) at 1, 2, 3, 5, 24 h after the start of the experiments.

4.3. Do polyphagous species taste phytoecdysteroids with less intensity?

Host selectivity of phytophagous insects is generally assumed as being evolutionary driven by plant secondary chemicals, possessing either toxic or deterrent activities (Dethier, 1954; Dethier, 1980; Schoonhoven et al., 1992). As a correlate, it was proposed that deterrent cells are less sensitive in polyphagous insects than in monophagous or oligophagous insects (Bernays and Chapman, 1994; Schoonhoven et al., 1998). This proposition was used as a tentative model for explaining host plant shifts in Lepidoptera (van Drongelen, 1979; Bernays and Chapman, 1994; Schoonhoven et al., 1998). It was recently challenged by observations on sister species differing by their diet breadth but exhibiting no consistent differences in their perception of deterrent compounds (Bernays and Chapman, 2000).

Our observations do not fit with this hypothesis although they do not represent a rigorous test of it. The high sensitivity to 20E and the behavioural deterrentcy found in *B. mori* conforms to the prediction because this species is oligophagous. In *S. littoralis*, the hypothesis predicts less sensitive receptors. This might be the case if one considers that responses to 20E are less intense than for example in *B. mori* both in terms of detection threshold (10^{-5} vs. 10^{-6} M) and in the steepness of the dose–response curve.

However, the high sensitivity recorded in the taste sensilla of *O. nubilalis* larvae was not expected. This

insect species is considered as highly polyphagous, with more than 200 host-plants recorded (Hodgson, 1928; Manojlovic, 1984). Further studies are certainly needed to evaluate if these host plants are devoid of phytoecdysteroids, and to what extent plants producing phytoecdysteroids are actively avoided by *O. nubilalis* in the wild. Its main host plant, *Zea mays*, does not contain detectable levels of phytoecdysteroids (Devarenne et al., 1995).

4.4. Are phytoecdysteroids protecting plants?

Although the above evidence supports the hypothesis that phytoecdysteroids might help plants to deter polyphagous insects, the situation is not so clear. For example, many larvae of noctuid species (like *S. littoralis*) detoxify very efficiently large quantities of phytoecdysteroids in their diet. They do not need deterrent receptors to detect phytoecdysteroids in their food plants, unless detoxication of phytoecdysteroids presents a high metabolic cost. Even in a monophagous species like *B. mori* for which 20E is toxic (Tanaka, 1995) and E induces supernumerary ecdyses (Tanaka et al., 1994a), its deterrent receptors are only sensitive to 20E and not to E.

Nevertheless, it seems that the number of insects detecting phytoecdysteroids might be greater than previously thought. In addition to *B. mori*, cells responding to 20E have been found in the oligophagous larvae of *P. brassicae* (Ma, 1969), of *Pieris rapae* (van Loon and Schoonhoven, 1999), of *Mamestra brassicae* (Descoins and Marion-Poll, 1999), and in two polyphagous species *O. nubilalis* (this paper) and *Lobesia botrana* (Mondy et al., 1999). Likewise, the extensive feeding test studies conducted by Dinan and Blackford suggest that larvae from several oligophagous species actively avoid phytoecdysteroids (Blackford and Dinan, 1997a; Blackford and Dinan, 1997b; Dinan, 1998). This suggests that sensory perception of phytoecdysteroids and the corresponding avoidance behaviour have some adaptative component. This subject clearly deserves more work to ascertain if this sensory perception is specific to phytoecdysteroids and is really mediated by deterrent cells.

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