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DOI
10.1046/j.1570-7458.1998.00316.x

Publication date
1998

Document Version
Final published version

Published in
Entomologia Experimentalis et Applicata

Entomologia Experimentalis et Applicata **87:** 143–155, 1998.

Chemoreception of oviposition inhibiting terpenoids in the diamondback moth *Plutella xylostella*

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Accepted: January 22, 1998

Key words: Plutella xylostella, oviposition inhibitor, contact chemoreceptor, morphology, electrophysiology, ablation, terpenoids, sensory mechanism

Abstract

The effects of six terpenoids and two terpenoid containing extracts of the neem tree (*Azadirachta indica* A. Juss.) on oviposition by the diamondback moth (*Plutella xylostella* L., Yponomeutidae: Lepidoptera) were tested. Two drimane terpenoids, the sesquiterpenoid polygodial and the neem extract Margosan-O exerted significant inhibitory effects at the dosages tested. Ablation experiments showed that both antennae and fore-tarsi contributed to mediation of the inhibition by a drimane. Location of chemosensilla on prothoracic tarsi and ovipositor was examined by scanning electronmicroscopy. Electrophysiological recordings from ovipositor and tarsal taste sensilla showed that distilled water produced distinct responses from one neuron. In tarsal sensilla, ethanol and drimane solutions produced responses from two neurons, one of which might be the water cell that fired at a reduced rate. A drimane significantly decreased the responses of tarsal chemoreceptors to a cabbage leaf extract, which is a possible sensory mechanism leading to behavioural avoidance of this compound.

Introduction

The diamondback moth *Plutella xylostella* L. is a world-wide pest of most crucifers. Chemical control of *P. xylostella* has become less effective because of its high ability to develop resistance to almost all groups of insecticides (Talekar, 1992; Shelton et al., 1993). Additional management strategies, such as the use of oviposition inhibitors, might retard the development of such resistance. Terpenoids constitute a group of secondary plant substances playing an important part in plant defense against phytophagous insects. Azadirachtin, a tetranortriterpenoid occurring in *Azadirachta indica* (Meliaceae), is a well-documented example of a secondary plant compound with deleterious effects on behaviour and physiology of a broad range of insect species (Mordue, 1994). Another tetranortriterpenoid, toosendanin, isolated from *Melia toosendan* (Meliaceae) showed strong antifeedant, toxic and oviposition inhibitory effects on a range of insects (Chiu, 1985). Terpenoids with simpler molecular structure and high bioactivity likewise hold promise as plant protection agents. Polygodial is a drimane sesquiterpenoid isolated from water-pepper *Polygonum hydropiper* as well as from *Warburgia ugandensis* and *W. stuhlmannii* and possesses antifeedant activity against a number of lepidopterous species (Blaney et al., 1987; Schoonhoven & Yan, 1989; Messchendorp et al., 1996). The effects on *P. xylostella* oviposition of six pure terpenoids and two preparations rich in terpenoids were assessed using a dual-choice bioassay.

Despite its economic importance (Talekar, 1992), only recently a detailed study of *P. xylostella* oviposition behaviour was published (Justus & Mitchell, 1996). Contact stimuli mediated by antennal and ovipositor sensilla were found to exert a dominant effect on triggering oviposition behaviour. The role of tarsal sensilla, however, was not addressed. Furthermore, physiological studies of contact chemoreception of semiochemicals by *P. xylostella* are lacking in the

literature. The latter two aspects are addressed here. Ablation bioassays were conducted to determine which sensory organs were responsible for mediating the oviposition inhibitory effect of a drimane compound. Contact chemoreceptors present on the ovipositor and tarsi were studied morphologically and electrophysiologically to gain understanding of the sensory mediation of inhibition.

Materials and methods

Chemicals. Azadirachtin (>99%) was obtained from Dr E. D. Morgan, Chemistry Department, Keele University, U.K.; Margosan-O (azadirachtin content 0.3%) was provided by Ir. A. Hissink, Sierra Chemical Europe; NeemAzal (azadirachtin content 44%) was purchased from Trifolio-M (Lahnau, Germany); toosendanin (98%) was made available by Dr Luo Liner, Biology Department, Beijing University; Ursolic acid (>90%) was purchased from Sigma Chemical Co. Drimane A, B and polygodial (Figure 1) were synthesized by Dr. B. J. M. Jansen, Department of Organic Chemistry, Wageningen Agricultural University. Chemicals were dissolved in ethanol and stepwise diluted with distilled water (1:10–1:100, depending on solubility).

Oviposition bioassay. Inhibitory effects were quantified using a dual-choice behavioural bioassay. In a 22 mesh gauze cylinder (⊘ 9 cm, length 20 cm), five females were offered filter paper discs (⊘ 9 cm) presented at both ends. Cabbage (*Brassica oleracea* L. var. *gemmifera* cv Titurel) leaf juice was prepared by grinding cabbage leaves together with water or ethanol (1 g/3 ml) and applied to both paper discs by dipping them in the cabbage leaf juice and subsequent drying at room temperature. One of these paper discs was used as control (C) while at the opposite side (treatment, T) 1 ml solution of test material was sprayed with a chromatography sprayer evenly on the paper disc previously saturated with cabbage leaf juice. After 24 h at 25 °C, (L16:D8), eggs laid on C- and T-discs were counted and the inhibition index was calculated as (C−T)/(C+T).

Direct observation of female oviposition behaviour. The behavioural responses of females to drimane A were observed after the females were put into the cylinder during the light period by slightly tapping the cylinder to elicit flight behaviour. Behaviour upon landing on the filter papers at either side of the cylinder was recorded.

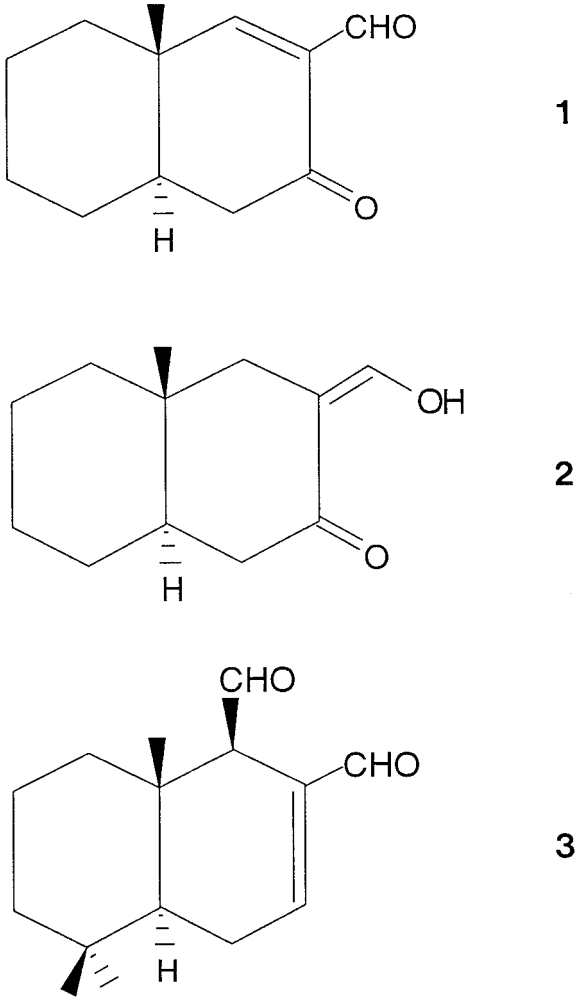

Figure 1. Chemical structures of drimane A (1), drimane B (2) and polygodial (3).

Ablation bioassay. Two ablation treatments were investigated: group A: removal of both antennae; group B: removal of both antennae + inactivation of the sensilla on the prothoracic tarsi by treatment with 5M HCl for 40 s (Städler, 1977). During the operation, females were anaesthesized by CO_2. Sham experiments (results not presented) showed that treatment of the tarsi with distilled water did not affect the inhibition index, therefore control insects were only exposed to CO_2 for the same time as treatment groups.

Scanning electron microscopy. Prothoracic tarsi and abdomens of female moths were excised and air dried for 48 h at 37 °C, coated with a 200 Å layer of gold and viewed with a Jeol JSM 35C scanning electron microscope. To obtain a better view of the sensilla, some tarsal preparations were de-scaled by gentle rolling over sticky tape.

Electrophysiology. Tip-recording (Hodgson et al., 1955), modified as described by van Loon (1990) was used to record responses from chemosensilla on the tarsus and ovipositor of *P. xylostella*. The input bias current of the AD 515K (Analog Devices) operational amplifier used as pre-amplifier was 0.1 pA in order to prevent electrical stimulation (Maes, 1977). Recordings from tarsal sensilla were from the sensillum located medially between the claws ('pseudempodium'; Figure 2A). Leaf extract for electrophysiological testing was prepared by crushing cabbage leaf in the same weight of water and filtering with 'Weissband aschefrei' filter paper, after which the extract was diluted 50 times with distilled water. This dilution was necessary to prevent fast crystallization at the fine tip of the glass capillary microelectrode. Directly upon dilution, leaf extracts were kept on ice until use to prevent decomposition. Drimane A was dissolved in ethanol 98% and subsequently diluted in distilled water, yielding a final concentration of 1 mM drimane and 0.1% ethanol. Electrophysiological responses were quantified using the number of spikes in the first second after the start of stimulation. Neural activity was sampled with an Intel 486 based personal computer equipped with a Metrabyte DAS16 A/D conversion board. An interface was used ('Go-box') for signal conditioning. This involved a second order band pass filter (-3 dB frequencies: 180 and 1700 Hz). Digitized traces were analyzed by means of Sapid Tools v. 3.5 software (Smith et al., 1990). ANOVA and the Student–Newman Keuls multiple comparison test were used for detecting differences of effectiveness between stimuli. During the experiments reported in Tables 2 and 3, all sensilla were tested with all solutions. The different replicate numbers are explained by the circumstance that not in all cases the recordings could be analyzed, due to poor signal-to-noise ratios.

Results

Oviposition bioassay. Two drimanes (A and B), polygodial, and the neem extract, Margosan-O, significantly inhibited oviposition at the doses tested (Table 1). Azadirachtin, a major triterpenoid in Margosan-O, was inactive as a pure compound at the concentration equivalent to that present in 5% Margosan-O. Among the materials tested drimane A and polygodial had the highest inhibitory activity. Ursolic acid was found to stimulate oviposition. The oviposition rates found (7–18 eggs/female/24 h) in response to the aqueous *B. oleracea* homogenate were similar to those found by Reed *et al.* (1989; 9–29 eggs/female/scotophase, depending on the crucifer species tested), using a similar filter paper substrate bioassay.

Direct observation of female behaviour. When females walked on the surface of the drimane treated filter paper, they left the filter paper after an average of 5 s, stopped walking and started extensive grooming of their antenna with their prothoracic legs, followed by rubbing the latter over the labial palps. This sequence of behaviours was not observed for females alighting on the control side. Females always used their ovipositor to touch the surface prior to laying eggs. In order to assess the contribution of different organs bearing chemosensilla, we performed ablation experiments.

Ablation bioassay. In the ablation experiments, the average inhibition index (0.569) of the A-group from which both antennae were excised was significantly lower than for the control group (0.737, $P < 0.05$ (Wilcoxon's matched pairs signed rank test, one sided)). The average inhibition index for the group with both antennae excised and prothoracic tarsal chemosensilla chemically ablated (0.385) was significantly lower than for the control group (0.786, $P < 0.005$). It was also significantly lower than the inhibition index of the A-group ($P < 0.025$). Yet after combined ablation of antennae and fore-tarsi, there was still a significant inhibitory effect of drimane A compared to the control group ($P < 0.05$). The attempted combined ablation of antennae and all six tarsi caused over 50% of mortality of the treated females and was not pursued further.

Morphology of tarsal and ovipositor sensilla. The tarsi and ovipositor were investigated by scanning electron microscopy (Figure 2). With the exception of a small area on the ventral side of the fifth tarsomere, the tarsal segments are covered with scales. Sensilla are present on the ventral surface and their tips just penetrate through the scales. In general each segment bears four blunt tipped sensilla, two in a lateral and two in a more ventral position. The fifth tarsomere shows

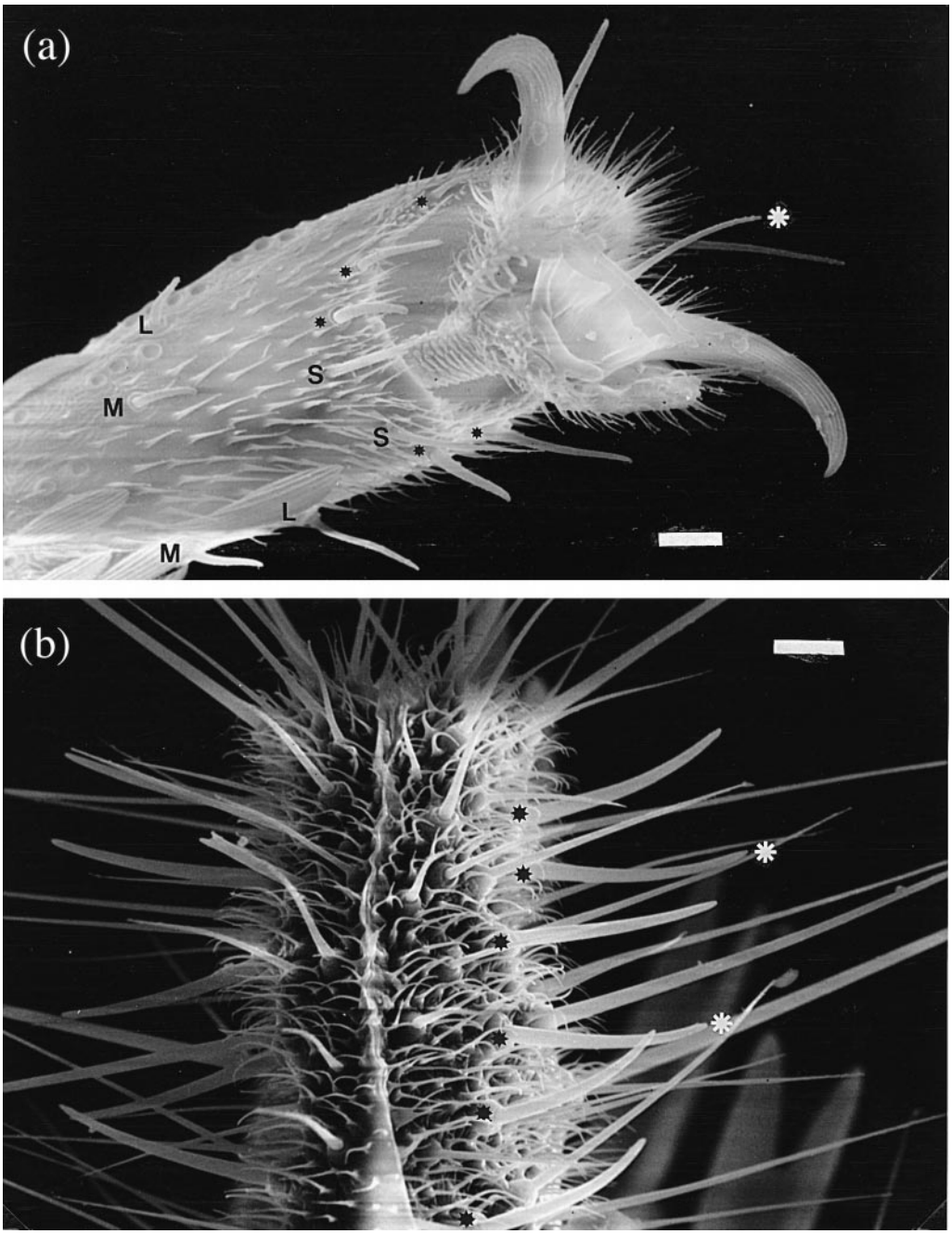

Figure 2. Scanning electron micrographs of a – ventral side of the 5th tarsomere of a prothoracic leg of a *P. xylostella* female showing the 'pseudempodium' sensillum (tip marked with white asterisk) medially between the pulvilli. Different sensilla are indicated by capital letters at their base: L – lateral taste sensillum; M – medial taste sensillum (two L- and two M-sensilla occur on each tarsomere); S – sharp-tipped sensillum (probably tactile); black asterisks mark the bases of taste sensilla along the distal edge of the 5th tarsomere; five out of six normally present are seen in this view (see also text). b – ventral view of taste sensilla along the opening of the ovipositor of *P. xylostella*. Black asterisks mark the sockets of blunt-tipped taste sensilla, six in this case. White asterisks mark the tips of sensilla #2 and #4 from which most electrophysiological recordings were obtained. White bar represents 10 μm.

Table 1. Results of dual choice oviposition bioassays to establish possible inhibitory effects of terpenoids and terpenoid containing extracts to *Plutella xylostella*. The Wilcoxon Matched-pairs Signed-ranks Test was used to assess the significance of differences. One ml of solution of the indicated concentration was sprayed on a 9 cm ⌀ filter paper disc on the treated side and 1 ml of solvent was sprayed on the control side. Treated and control discs had been pretreated with cabbage extract

Material tested	Conc.	#Rep[a]	Egg# treatment	Egg# control	Inhibition index (SD)[b]
Margosan-O	2.5%	15	361	767	0.401(0.283)**
Margosan-O	5%	15	75	434	0.527(0.127)***
NeemAzal	0.15%	6	268	261	−0.008(0.192)
Azadirachtin	0.27 mM	15	430	425	−0.058(0.105)
Toosendanin	5 mM	15	509	440	−0.128(0.10)
Polygodial	1 mM	15	261	524	0.385(0.078)***
Polygodial	5 mM	15	44	492	0.791(0.067)***
Drimane A	1 mM	15	192	413	0.327(0.058)***
Drimane A	5 mM	15	65	580	0.787(0.135)***
Drimane B	5 mM	14	144	367	0.430(0.1)***
Ursolic acid	2.2 mM	15	415	250	−0.244(0.127)*

[a] number of replicate cages; each cage contained five *P. xylostella* females.
[b] *: $P < 0.02$; **: $P < 0.01$; *** $P < 0.001$.

Table 2. Electrophysiological responses of ovipositor contact chemosensilla #2 and #4 of *Plutella xylostella* females

Stimulus	Response (spikes/s±SD)		n
Experiment 1			
Distilled water	41.4 ± 10.0	a	12
Ethanol 0.1%	54.7 ± 10.4	a	12
Drimane A 1mM (in 0.1% ethanol)	55.6 ± 10.1	a	12
Experiment 2			
Leaf homogenate	32.0 ± 4.3	a	22
Leaf homogenate/drimane A 1 mM	31.4 ± 3.1	a	22

Means followed by the same letter are not significantly different at $P < 0.05$. Comparisons are within the two experiments.

an additional row of six sensilla along the scaleless distal edge. This row consists of six blunt tipped contact chemoreceptors and a ventral pair of sharp-tipped sensilla. A single hair is present medially between the pulvilli ('pseudempodium' *sensu* Crampton, 1923). The chemosensory responses reported in this paper were all recorded from this sensillum.

The ovipositors are densely covered with long sharp-tipped sensilla, presumably mechanosensory in nature because it was not possible to obtain electrical contact via their tips. A row of 4–7 shorter blunt-tipped sensilla in pronounced sockets is present on each side of the ventral opening. The number of these contact chemoreceptors was observed to be somewhat variable (Justus & Mitchell, 1996). In a group of 90 individuals the frequencies of occurrence of 4, 5, 6 and 7 sensilla on each side were 1.1%, 30%, 66% and 3.4%, respectively.

Electrophysiology of ovipositor sensilla

Two of the 4–7 blunt tipped chemosensilla (normally the 2nd and the 4th chemosensilla as counted from the distal tip of the ovipositor) showed consistent responses to stimulation with the solutions employed. In some cases responses could also be obtained from

Table 3. Electrophysiological responses of the 'pseudempodium' tarsal contact taste sensillum of *Plutella xylostella* females

Stimulus	Response (spikes/s±SD)		n
Experiment 1			
Distilled water	49.0 ± 19.2	a	8
Ethanol 0.1%			
small amplitude cell	6.6 ± 3.3	A	5
large amplitude cell	24.6 ± 7.1	b	5
Drimane A 1 mM (in ethanol 0.1%)			
small amplitude cell	11.0 ± 2.9	A	3[a]
large amplitude cell	28.3 ± 11.5	b	9
Experiment 2			
Leaf homogenate	32.8 ± 4.9	a	12
Leaf homogenate/drimane A 1mM	23.6 ± 3.9	b	12

Means followed by the same lower case (for the cell producing a large spike amplitude) or capital letter (for the cell identified by its small spike amplitude) are not significantly different at $P < 0.05$. Comparisons are within the two experiments. [a]: in only 3 out of 9 cases, a second neuron firing a small amplitude spike was noted.

sensilla 3 and 5. In the sensilla 2 and 4, one neuron responded to distilled water (Figure 3; designated as water cell in the following). Ethanol and drimane elicited responses almost exclusively from one cell at spike frequencies similar to that recorded in response to water alone (Figure 3; Table 2). Leaf homogenate and the mixture of leaf homogenate and drimane resulted in similar response frequencies (Table 2).

The average response frequencies obtained from a neuron present in these two hairs to KCl solutions exhibited a negative dose-response relationship and were significantly reduced compared to those to water alone (Figure 4). While water elicited a response from one cell only, at doses higher than 0.05 mM KCl activities from a second and third cell, producing smaller spike amplitudes than the water cell, were recorded. A clear reduction of signal-to-noise ratio was noted at 0.05 mM and higher concentrations. At 1 mM and higher concentrations the signal amplitude declined drastically (Figure 5F). More concentrated KCl solutions (up to 25 mM) produced high response frequencies from at least three cells (Figure 5G). The total number of spikes was highest at 5 mM (average total frequency 150 spikes/s) and declined at 10 and 25 mM to a level of 100 spikes/s.

Electrophysiology of tarsal sensilla

The 'pseudempodium' sensillum located medially between the pulvilli (Figure 2A) possessed a cell responsive to distilled water. One cell and in some preparations two cells responded to 0.1% ethanol (the solvent for drimane), and 1 mM drimane (Figure 6). Although it appears that in response to ethanol and drimane the larger spike originates from the water cell, the recordings do not allow an unambiguous conclusion on the identity of the cells activated. Neither cell was significantly affected by drimane A, nor were other neurons activated. In a separate experiment, the response to a mixture of leaf extract (the oviposition stimulant mixture in the bioassay) and 1 mM drimane was found to be significantly reduced as compared to leaf extract alone (Table 3). In response to the latter stimuli, a typical unicellular response was recorded (Figure 7). The other sensilla on the tarsi did not significantly respond to any of the solutions tested, although electrical contact was established as apparent from the recordings that showed on average 5 spikes/s.

Discussion

Behavioural responses to terpenoids. The behavioural assays clearly demonstrated the potential of several terpenoid compounds to inhibit oviposition by *P. xyl-*

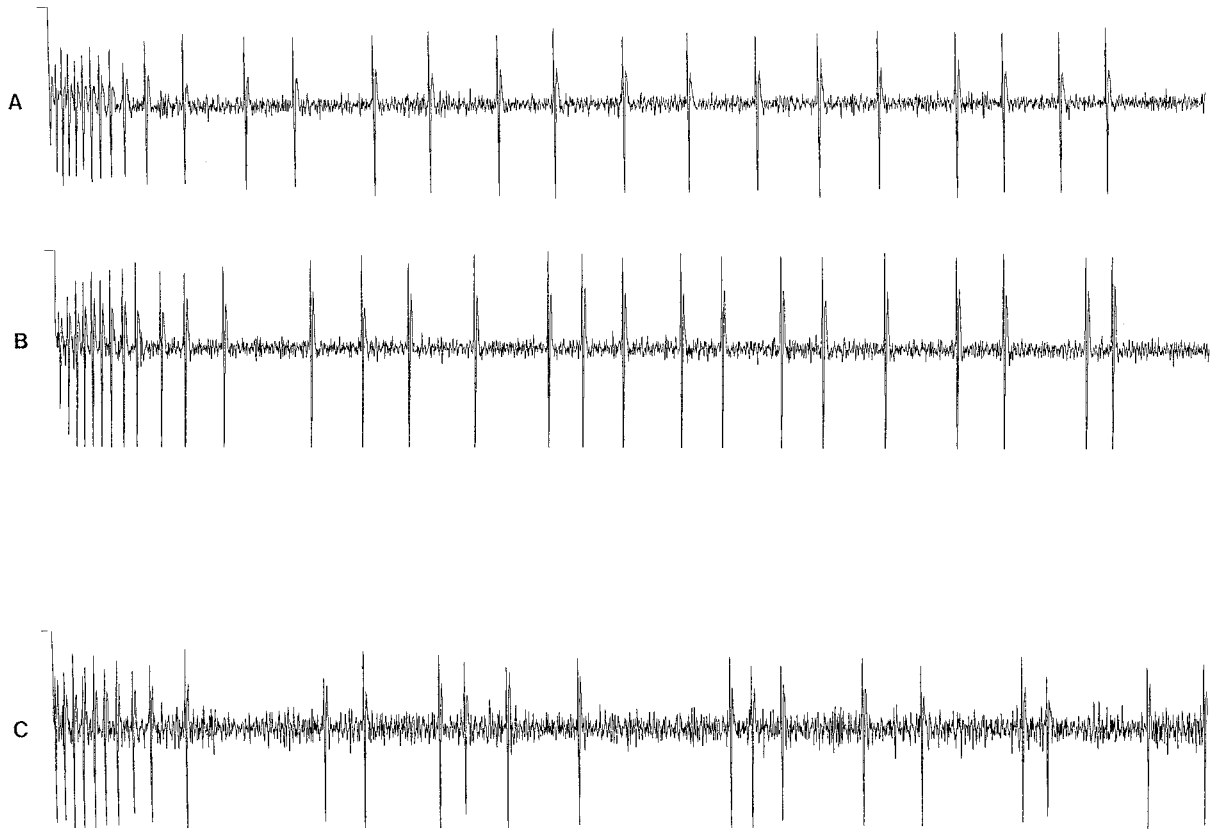

Figure 3. Electrophysiological responses (0–1000 ms) of a taste sensillum #4 on the ovipositor of *P. xylostella* (see Figure 2B). Stimuli: A: distilled water; B: ethanol 0.1% in distilled water; C: drimane A 1 mM in ethanol 0.1%.

ostella. When azadirachtin, a major active compound in the complex extract Margosan-O and in the purified preparation NeemAzal, was tested in pure form at a dosage equivalent to that present in Margosan-O and NeemAzal, no significant inhibition was noted. Surprisingly, the structurally much simpler sesquiterpenoid polygodial and drimanes were effective in pure form. Ursolic acid in our bioassay stimulated oviposition to a level significantly higher than that of the leaf extract alone. This triterpenol acid is structurally highly similar to amyrins, triterpenol compounds naturally occurring on the surface of cabbage leaves (Eigenbrode et al., 1991). The only difference is that ursolic acid has a carboxylic acid group instead of a methyl group at position 17 of the triterpenoid skeleton. Amyrins are antifeedants to *P. xylostella* neonate larvae (Eigenbrode & Espelie, 1995). The latter compounds occur in relatively large amounts on the surface of glossy *B. oleracea* genotypes resistant to *P. xylostella* neonates. On the other hand, some glossy genotypes are preferred over glaucous genotypes for oviposition by *P. xylostella* (S.E. Eigenbrode, pers. comm.). The dosage of ursolic acid assayed is about 15× that found for amyrins in the wax layer of the resistant cabbage NY 8329 (0.6 μg/cm^2; Eigenbrode et al., 1991). It is therefore likely that ursolic acid at natural concentrations in the wax layer has a stimulatory or neutral rather than an inhibitory effect.

Ablation experiments. The ablation experiments showed that both antennae and foretarsi conveyed information on the presence of drimane A, the inhibitor selected for these experiments. Females lacking information from antennae and prothoracic tarsi still showed significant avoidance of drimane A treated substrate. This indicates that other gustatory sensilla present on mid and hind-tarsi, ovipositor, proboscis and labial palps provide sufficient information to detect this inhibitor. As ablation of taste hairs on all tarsi, ovipositor, labial palps and proboscis is technically not

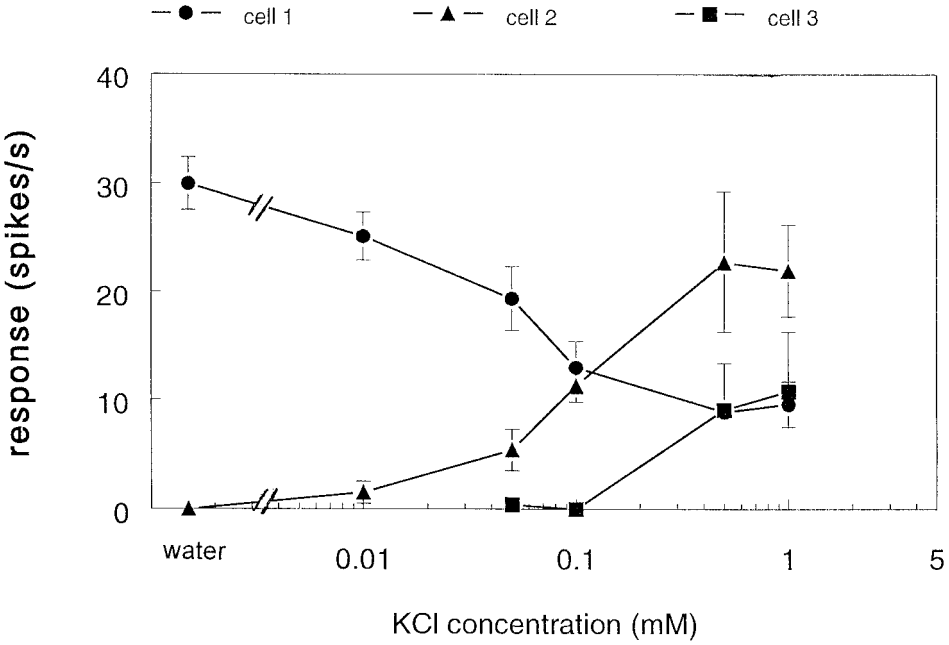

Figure 4. Electrophysiological dose-response curves of the response to water and five KCl concentrations of #2 and #4 ovipositor sensilla (results pooled). Mean results (± SEM) from 10 females on each of which all six stimulus solutions were tested to enable cell identification on the basis of template by comparing recordings taken from an individual female (see Smith et al., 1990).

feasible due to the concomitant high mortality, a role of the respective taste hairs located on these appendages could not be quantified.

Morphology. The structure of chemosensilla on the tarsi and ovipositor of the female *P. xylostella* have not been reported before. The distribution of the chemosensilla on the tarsi of *P. xylostella* was quite different compared to that of female *Pieris* butterflies which possess 14-15 contact chemosensory sensilla in a cluster associated with a conspicuous spine and a row of 5 laterally located taste hairs (Ma & Schoonhoven, 1973; Städler et al., 1995). Numbers and location of sensilla are highly similar to the moths *Yponomeuta cagnagellus* (belonging to the same family as *P. xylostella*; Roessingh et al., 1995), *Chilo partellus* (Waladde, 1983) and *Ephestia kuehniella* (Anderson & Hallberg, 1990). A situation intermediate between the butterflies and moths is represented by *Manduca sexta* (Kent & Griffin, 1990). In *Manduca sexta* the pseudempodium is innervated by 2–3 sensory neurons that extend into the lumen of the sensillum (Kent & Griffin, 1990). Although no pore was observed by these authors, they assumed one would be present in the barbed tip of the sensillum. The results presented in this paper show that the pseudempodium indeed has a chemosensory function, and might play a role in the assessment of host suitability (see below).

No clear responses could be recorded from the blunt-tipped sensilla on the ventral side of the fifth tarsal segment, although electrical contact was established. Interestingly, in *M. sexta* similar blunt tipped sensilla were reported to show a pitted surface (in addition to a sub-apical pore), suggesting an olfactory function (Kent & Griffin, 1990). If this is indeed the case, it might explain the reported failures to evoke clear responses to gustatory stimuli from these sensilla in *P. xylostella* and several other moth species (Anderson et al., 1993; Roessingh et al., 1995). On the other hand it might just as well indicate that the adequate stimuli were not applied in these cases.

Location and shape of the 4–7 chemosensilla on the ovipositor of *P. xylostella* were similar to that in the yponomeutid *Acrolepiopsis assectella* Zell., in which 7–8 putative contact chemosensilla of two types (4–5 'D' and 3 'E') have been distinguished (Faucheux, 1988). In other Lepidoptera the numbers of similar chemosensilla on the ovipositor varied between 10-17 in *P. brassicae* (Klijnstra & Roessingh, 1986) and 2 in *C. partellus* (Waladde, 1983).

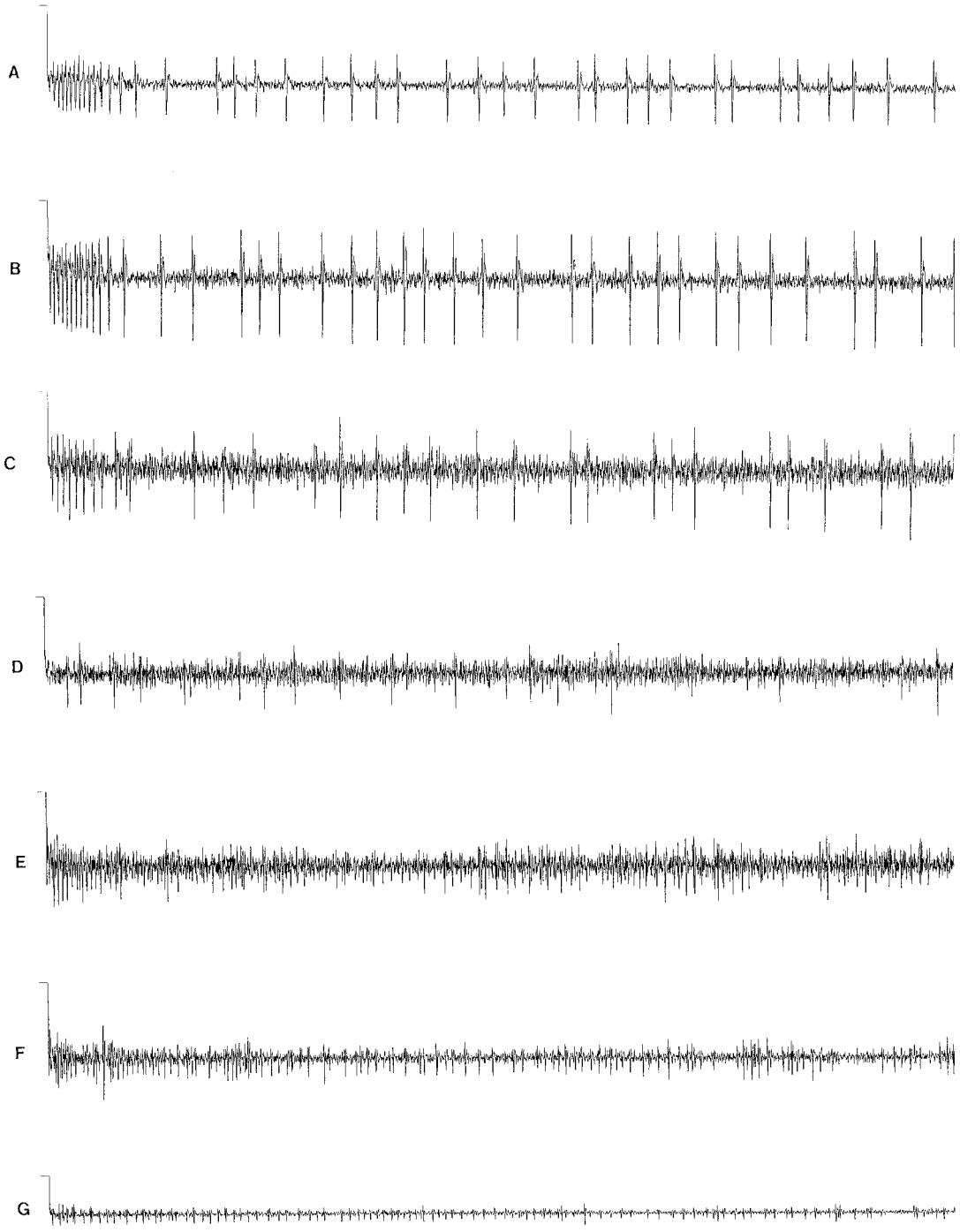

Figure 5. Electrophysiological recordings (0–1000 ms) from an individual ovipositor sensillum to water and increasing KCl concentrations. Amplification is identical for all 7 traces. A: distilled water; B: 0.01 mM KCl; C: 0.05 mM KCl; D: 0.1 mM KCl; E: 0.5 mM KCl; F: 1 mM KCl; G: 5 mM KCl.

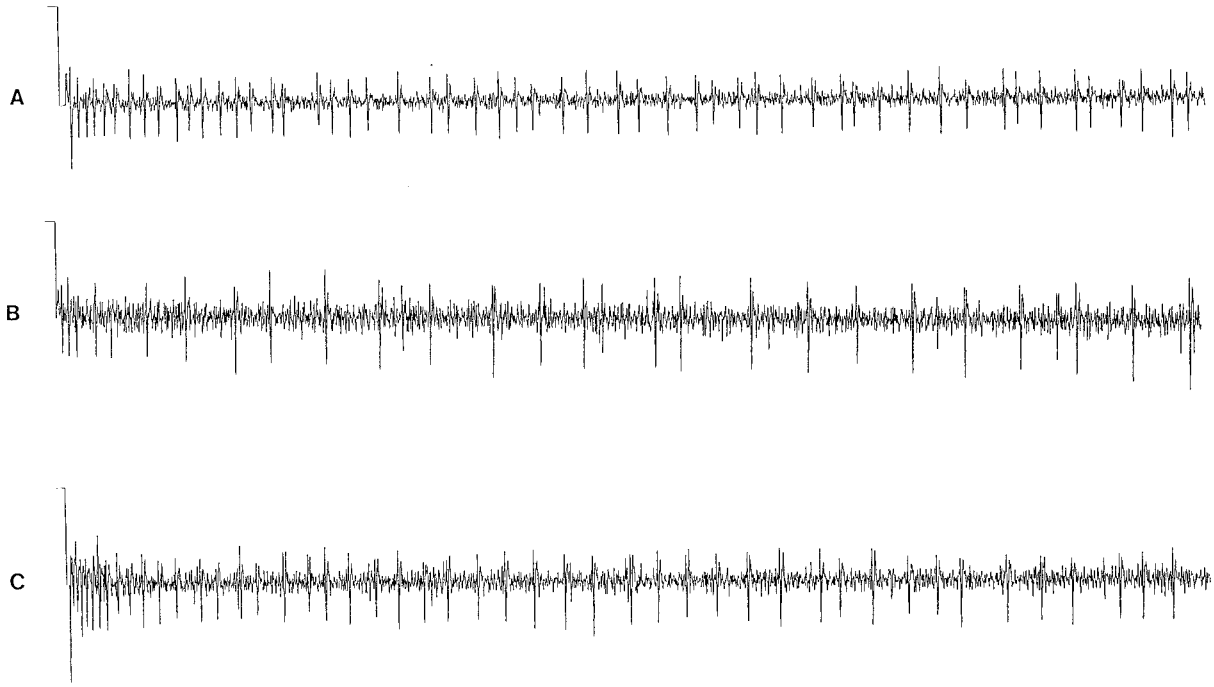

Figure 6. Electrophysiological recordings (0–1000 ms) from the 'pseudempodium' tarsal sensillum (see Figure 2A) in response to A: distilled water; B: ethanol 0.1.%; C: drimane A 1 mM in 0.1% ethanol.

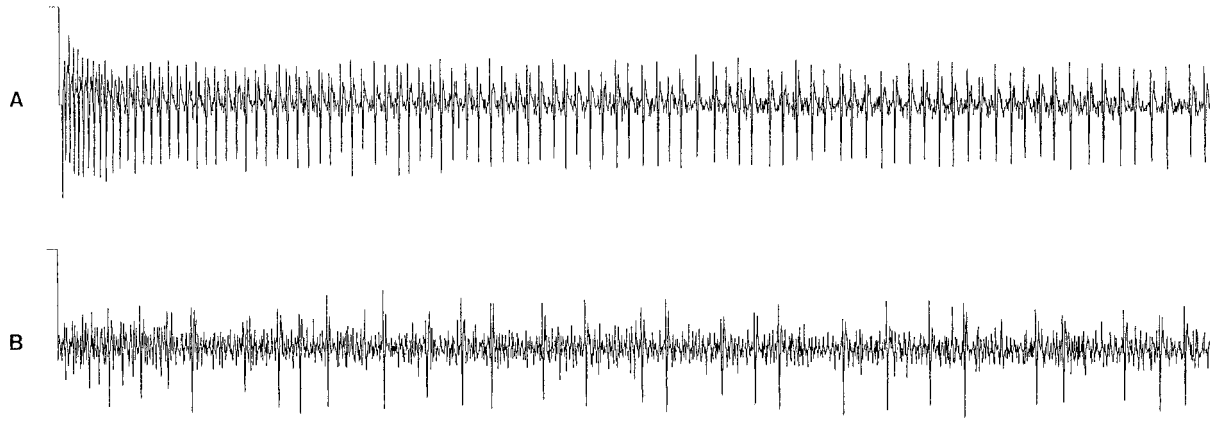

Figure 7. Electrophysiological recordings (0–1000 ms) from the 'pseudempodium' tarsal sensillum (see Figure 2A) in response to A: cabbage leaf homogenate; B: a mixture of cabbage leaf homogenate and 1 mM drimane A.

Electrophysiology: responses to water. Contacting the tarsal and ovipositor taste sensilla of *P. xylostella* females with distilled water resulted in a pronounced response of one neuron (Figures 4 and 6). In ovipositor sensilla, dilute KCl solutions caused a reduction in the firing frequency of this cell and reduced signal-to-noise ratio (Figures 5 and 6). At higher KCl concentrations, which were still lower than those commonly applied in electrophysiological experiments, at least three different taste neurons started firing. For these reasons we chose to offer the pure compounds in distilled water to increase the possibility of separating cell activities. In several saprophagous fly species, a water receptor cell has been found (reviewed in Dethier, 1976). The activity of water receptor cells is reduced in the presence of ions or dissolved organic compounds. Rees

(1970) found that potassium ions inhibit water receptor activity in the fly *Phormia terranovae* at concentrations above 10 mM KCl, i.e. at $>200\times$ higher KCl dosages than those found to affect water cell activity in *P. xylostella*. It has been suggested (Dethier, 1976) that water cells are in fact generalist ion receptors sensitive to very low electrolyte concentrations, the source of which would be the receptor lymph. The chemosensory cells in the ovipositor sensilla seem quite sensitive to KCl as at concentrations higher than 0.1 mM several cells are activated (Figure 5). No other examples of water cells in moths are known to us. In phytophagous insects water cells have rarely been described. An exception is the fly *Delia radicum* L. in which indications for a water receptor were found in tarsal 'B'-sensilla (Städler, 1978; P. Roessingh, unpubl.). In our study care was taken to minimize the duration of contact with stimulus solutions. This was done to prevent leakage of endogenous ions from out of the dendritic space. Such leakage may cause depletion of ions and has been implied as a cause of increased and decreased responses to dissolved stimuli (den Otter, 1972; Broyles & Hanson, 1976).

It is not entirely sure to what extent the water-sensitive neuron is activated by ethanol, drimane A or leaf homogenate. In the tarsal sensillum ethanol and drimane activated two cells. Based on its amplitude, one of these might be the water cell, the response of which in that case was significantly inhibited (Table 3; Figure 6). In the ovipositor sensilla predominantly one cell was firing when ethanol or drimane A dissolved in water were offered.

Regarding the effect of water in the bioassay, it was noted that a higher water content of filter paper discs during our bioassay resulted in higher oviposition rates. As filter paper water content during the assay was difficult to control, no further attempts were made to quantify this. Although leaf surfaces are waxy and dry, increased oviposition rate in response to higher humidity might be functional in view of the fact that in the leaf boundary layer surrounding the waxy leaf surface, air humidity is known to be higher (Willmer, 1986).

Chemosensory coding of inhibition. To elucidate the sensory mechanisms operating in the detection of inhibitors we focussed on the tarsal and ovipositor taste sensilla. We found no evidence for the presence of a separate neuron sensitive only to inhibitors, a so-called deterrent receptor, analogous to those found in several species of *Pieris* butterflies (Städler et al., 1995; Du et al., 1995). Instead, the sensory effect of the inhibitor tested was the reduction of the firing frequency of a neuron responsive to leaf extracts, an example of peripheral interaction (van Loon, 1990, 1996; Schoonhoven et al., 1992; Shields & Mitchell, 1995). This phenomenon occurred in the tarsal taste hairs, but was not detected in the ovipositor taste sensilla. Oviposition inhibition in *Heliothis virescens* was suggested to be due to peripheral interaction of the inhibitory compound with a sucrose sensitive taste neuron, based on electrophysiological experiments on ovipositor taste hairs (Ramaswamy et al., 1992). Taste sensilla on the tarsus have been shown to mediate information crucial for stimulation and inhibition of oviposition behaviour in Diptera and several day-foraging Lepidoptera (reviewed by van Loon, 1996). Regarding moths, only a few preliminary behavioural and electrophysiological studies on the role of tarsal taste perception are known (reviewed by Ramaswamy, 1988). The role of chemosensilla on the antenna was studied in detail for oviposition by *Spodoptera littoralis* (Anderson et al., 1993). Oviposition is repelled by conspecific larval frass. A mixture of six frass-derived compounds, benzaldehyde and five terpenes, was found to evoke avoidance behaviour, i.e. the compounds acted as repellents. Seven types of receptor cells specifically sensitive to frass-derived compounds were identified in olfactory sensilla on the antenna. Olfactory neurons highly specific to single aromatic terpenes were found. This paper documents the first attempt to study in some detail the contact chemosensory basis of oviposition inhibition in adult moths. In contrast to the situation in butterflies, no deterrent neuron was found. The responses to water found in *P. xylostella* taste sensilla have not been reported for lepidopteran taste sensilla. Most gustatory sensilla studied did not respond to the stimuli offered. In nature the contact chemoreceptors of *P. xylostella* females encounter apolar waxy leaf surfaces during exploration of potential plants for oviposition. In *P. xylostella* the combination of apolar compounds like alkanes and the polar glucosinolates, both present in cabbage wax layers (van Loon et al., 1992) enhances oviposition significantly compared to that of glucosinolates alone (Spencer, 1996). The use of polar solvents such as water to offer potential taste stimuli may lead to sensory responses different from those to apolar substrates. A real step forward in studies like the present would therefore be to record from taste sensilla contacting apolar substrates.

Acknowledgements

We thank André Gidding for rearing diamondback moth, Wim Frentz, Rieta Gols and Lindy Messchendorp for technical assistance and advice. We gratefully acknowledge the funding provided by the State Science and Technology Commission of the People's Republic of China and the Koninklijke Nederlandse Akademie van Kunsten en Wetenschappen to the first author.

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