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A COMPARATIVE STUDY OF HOST RECOGNITION AND THE SENSE OF TASTE IN *LEPTINOTARSA*

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Summary

Three species of *Leptinotarsa* beetles (*L. decemlineata*, *L. texana* and *L. haldemani*) with different host plant specificity were studied using behavioural and sensory physiological measures in an attempt to determine whether responses from chemosensory cells in the galeal sensilla of adult beetles vary with host plant preference in an understandable manner. Saps from leaves of *Solanum tuberosum*, *S. elaeagnifolium*, *S. dulcamara* and *Lycopersicon esculentum* were used as sensory stimuli. Behavioural observations were made on newly emerged adults and approach time, exploration time and number of bites in the first minute of the first meal were recorded. Number of bites in the first minute of the first meal differed across the four plant species for the three beetle species. For *L. decemlineata* and *L. texana*, sensory responses from cells in the galeal sensilla showed differences across plant species that could be related to host preference. It is suggested that at least two general types of sensory coding may be involved in host recognition by beetles in this genus. First, a 'coarse-grained' code based on degree of variability of input may operate to help the insect distinguish non-solanaceous plants from solanaceous ones. Second, a 'fine-grained' code may help distinguish host from non-host within the family Solanaceae or perhaps within the genus *Solanum*. This fine-grained code may have elements of both the labelled line and across-fibre pattern codes discussed in the literature.

Introduction

The sense of taste can be pivotal in host recognition and in rejection of non-hosts by phytophagous insects. Often, herbivorous insects will approach a number of non-host plants, only to reject them upon closer examination, involving sample tasting or even limited ingestion. Such rejection may not necessarily involve deterrents, that is the lack of 'host-like' stimuli may be sufficient to cause rejection after sampling (Mitchell, 1988). Given the importance of gustation in host recognition, one would expect congeneric insect species with differing degrees of host fidelity to display sensitivity differences at the level of their gustatory cells. This would be

Key words: *Leptinotarsa*, Colorado beetle, gustation, gustatory coding, feeding behaviour, host plant selection, host plant recognition, taste.

particularly true if the experimental stimuli involved were the actual saps of host and non-host plants. We used this rationale in designing the experiments reported in this paper. Studies of plant–insect interactions tend to emphasize either physiological or behavioural components. In this study, we attempt to integrate these factors and compare them across three species in the hope of gaining a better understanding of host choice in a major pest insect and two closely related species.

From among 31 species of *Leptinotarsa* three were selected on the basis of host associations, availability and relative ease of rearing (both plants and insects). Guidance on host plant differences among *Leptinotarsa* species was obtained from Jacques (1988) and Hsiao (1974, 1976). T. H. Hsiao (personal communication) also made valuable suggestions regarding suitability of plants and beetles for laboratory colonization. The three species used were *Leptinotarsa decemlineata* (Say), *L. haldemani* Rogers and *L. texana* (Schaeffer), each restricted to feeding on certain members of the Solanaceae.

On the basis of larval feeding and development, Hsiao (1974) ranked *L. decemlineata* as most polyphagous of the three beetle species, *L. haldemani* as moderately host-specific and *L. texana* as highly host-specific. Other descriptions of host associations, based on collection data, can be found in Jacques (1988).

Four solanaceous plant species were chosen to cover the spectrum of larval feeding acceptability described in the literature, with particular reference to Hsiao (1974). These were *Lycopersicon esculentum* Mill., (tomato) *Solanum tuberosum* L., (potato) *S. dulcamara* L., (bittersweet) and *S. elaeagnifolium* Cav. (silverleaf nightshade). Interactions range from unacceptable as hosts (*L. haldemani* on *S. elaeagnifolium*) to solely acceptable as a host (*L. texana* on *S. elaeagnifolium*). Fig. 2 (bottom) is an adaptation from Hsiao (1974) showing the food spectra of larvae of the three beetle species on the four plant species.

Behavioural analysis was necessary to provide a preference ranking of hosts by adult beetles, much of the literature cited above having concerned larvae. Using detailed observations of adults from three populations of *L. decemlineata*, Harrison (1987) determined that, in its earliest stages, feeding on primary host plants follows a stereotyped sequence. Less acceptable plants evoke a variety of different behaviour patterns which can be interpreted as interruption and re-initiation of the pattern evoked by the primary host. Focusing on such short-term behaviours associated with the initial stages of physical encounter with a plant reduces confounding effects of progressive satiation and possible malaise due to toxic compounds found in non-host leaves.

The galeal sensilla are the best understood of the gustatory organs in *L. decemlineata* (Mitchell, 1988, and references therein). Their sensitivity to sucrose, γ-aminobutyric acid (GABA) and L-alanine and the modulation of responses to these compounds by alkaloids suggest that these sensilla are intimately involved in plant recognition. In addition, using leaf saps as stimuli, Mitchell *et al.* (1990*a*) showed that saps of host plants can evoke simple and reproducible responses from these multi-celled sensilla, while saps of non-hosts variously stimulate several of the cells in each sensillum, sometimes with high variability. It was of particular

concern to us to determine if this response pattern held across the three beetle species.

Since other mouthpart chemosensilla are present (Sen, 1988), we assume that the adult beetle uses many more sensory inputs to recognize its host than those provided by the galeal chemoreceptors, and that redundancy is built into the system. Consequently, it would not be surprising if such sensorially endowed animals could make normal food choices even without benefit of these sensilla. Conversely, if all chemosensory input apart from the galeal sensilla were removed, we doubt that the animal would have sufficient information to make natural food choices. Our goal in this comparative study was not to show that input from galeal sensilla is sufficient for host recognition. Rather, since these sensilla are almost certainly involved in host recognition in the whole animal, we expected that homologous sensilla from the three beetle species would show differences in the pattern of their responses to the different plant saps which in some way reflect their different host affinities. If such differences in pattern can be interpreted properly, then progress can be made towards understanding the sensory codes that underlie differences in host recognition among congeneric species with similar phylogenetic constraints.

Materials and methods

Insects and plants

Beetles for rearing stock were obtained from the field as larvae and/or adults. *L. decemlineata* were collected from potato in the Edmonton area; *L. haldemani* from *Physalis* spp. in southern Arizona and *L. texana* from *S. elaeagnifolium* in Hildago County, TX. Field beetles were added annually. Breeding adults were kept in aquaria to which fresh leaf material was added every other day. For all stages, photoperiod was maintained at 16 h:8 h L:D and temperature at 25±1 °C. Lighting was provided by full-spectrum fluorescent fixtures. Eggs were collected from leaves in the breeding aquaria and held in Petri dishes until hatching, or just prior to hatching. Neonate larvae, along with egg chorions, were transferred to bouquets of host leaves and stems in special 'nurseries' fashioned from two plastic food containers telescoped together. The top container had a hole in its bottom through which stems of plant cuttings could be pushed to reach a water reservoir contained in the bottom container. Third-instar larvae were harvested as necessary from the nursery and placed in small aquaria which had a 3–5 cm layer of sand and peat in the bottom. Fresh plant cuttings were placed in these aquaria for larval food and pupation occurred in the sand/peat. Emerging adults were harvested from these aquaria for experimental purposes and for the breeding colony. Twice daily harvesting of adults ensured that we were dealing with newly emerged insects for both behavioural and electrophysiological experiments. Additional details of the rearing methods can be found in Mitchell and Harrison (1984).

Plants used for rearing were *S. tuberosum* (var Norland), *S. dulcamara*, *L. esculentum* (var. Earliana) and *S. elaeagnifolium*. The potatoes were grown from

tubers, *S. dulcamara* and *L. esculentum* from seed obtained commercially and *S. elaeagnifolium* from wild Texas seeds. All plants were greenhouse-grown with the aid of high-intensity lighting for 16 h per day. Fertilization was carried out as needed, on average once per week (20:20:20; N:P:K). Greenhouse temperatures ranged from 28°C (heat of summer) to 18°C (winter nights). *S. tuberosum* were normally offered to *L. decemlineata*, though this was supplemented with *S. dulcamara* when potato stock was low. *L. haldemani* was offered *L. esculentum*, *S. dulcamara* and *S. tuberosum*, often all three simultaneously. *L. texana* was fed exclusively on *S. elaeagnifolium*.

Behaviour

Bioassays of several minutes as opposed to several hours provide more accurate measures of the sensory basis of plant–insect interaction. The use of adult beetles with no previous feeding experience also provides a better approximation of differences in any innate neural template (*sensu* Dethier, 1982) important in host recognition.

Adult beetles of all three species were used for behavioural analysis 4–24 h after emergence. Observations were made during the third to sixth hour of photophase to minimize any effect of diel periodicity. Overhead growth lights provided fairly uniform background illumination of the test area while a fibre optic light source was directed at the test leaf (see below) to provide a directional cue and to enable close observation of the mouthparts. A small fan directed air across the test leaf towards the beetle, providing a second directional cue, olfactory cues and standardizing the airflow conditions in the test area. Temperature during tests was maintained at 25±0.5°C and humidity ranged between 40 and 60 %.

A single beetle was placed on a Teflon rod of 1 cm diameter, approximately 25 cm from the end of the rod nearest the light and fan. This end of the rod also held a freshly removed leaf (undamaged except for the cut at the petiole) by means of a slot cut in the rod. Each beetle was tested with leaves from each of the four plant species separately. Presentation of leaves from each species was varied haphazardly and the order recorded. Beetles that fed in a given test were allowed to do so for only 60 s, thus satiation during the four-part test was unlikely. Effects of presentation order were not significant, supporting our assumption that order of presentation and amount consumed, in cases where a suitable plant was offered early, were not unduly influencing the results. This lack of significance also suggests that short-term memory, which could be important in such a repeated design, was not creating a bias. *P* values from analysis of variance (ANOVA) for effect of presentation order were 0.45 for approach time, 0.733 for exploration time and 0.967 for number of bites in 60 s, indicating no effect. (See below for an explanation of behavioural categories.) Test leaves or leaflets were selected from healthy plants. The petiole or petiolule was cut with a razor blade and placed in a vial containing tap water to maintain leaf turgidity. Similar-sized leaves and leaflets were chosen whenever possible, and each was used only once. Further standardization of leaf tissue area could have been achieved by cutting larger

leaves, but we avoided this on the assumption that it would introduce further variability. Since rate of locomotion proved to vary with beetle species but not with leaf species (see Results), the range of leaf areas presented was not as important as the damage effect might have been.

Observations were made through a one-way mirror to reduce the startle effect of the experimenter as she drew close to observe details of feeding. A hand lens was used when necessary. The beginning of a trial was determined when any part of the beetle passed a mark on the Teflon rod 10.8 cm from the leaf edge. At this point a timer was started. Time to the nearest second was recorded from the time the mark was passed until the leaf was touched by any part of the beetle, usually an antenna or fore tarsus. This was designated 'approach time'. 'Exploration time' was the time elapsed between the first contact and the first bite, with bite defined as squeezing the leaf with the mandibles. If individuals did not bite within 3 min of touching the leaf, they were assigned an exploration time of 180 s and that test was terminated. Animals that did bite were observed for 60 s following the first bite and the number of bites in this minute were counted. Thus, a beetle that approached the leaf and touched it but did not bite was assigned an approach time of 180 s and a bite count of 0. Time between being offered a different plant species varied by 3–10 min, depending on the individual beetle's overall activity. All times were measured by a Mountain clock timer installed in an Apple IIe microcomputer, and the bite count was entered in the computer at the end of each run. The computer generated a summary for each experiment which was used as the primary data source for subsequent analyses.

Statistical analyses were accomplished using SPSS.X (Nie *et al.* 1975) and UANOVA as a user-defined procedure (Terry Taerum, University of Alberta). Analyses used a repeated-measures ANOVA with individuals nested within beetle species crossed with plant species. The beetle species had unequal sample size. Data were not normalized because of the large sample size and reliance on ANOVA. TukeyB (Tukey's alternative procedure) was used as an *a posteriori* contrast test with $\alpha=0.05$.

Sensory physiology

Galeal contact chemosensilla described by Mitchell and Harrison (1984) were selected for study using leaf saps from the same plant species discussed above as the stimuli. Newly emerged adults were collected twice daily and set aside in plastic Petri dishes. Beetles were individually marked for the behavioural studies, following which they were placed in Petri dishes containing damp tissues, one beetle per dish. They remained in these dishes, in culture room conditions but with no access to plants, for 24 h following the behaviour tests. A random sample of individuals was chosen each morning for electrophysiological recording.

Preparation for recording was as described in Sutcliffe and Mitchell (1982) and Mitchell and Harrison (1984). This involved restraining the beetle with minimal physical damage, the only penetration of the body being made by the small pin inserted into the abdomen as a reference electrode. The tip-recording method was

used throughout, with the stimulating/recording electrode containing plant sap. The signal was amplified with a clamping pre-amplifier (George Johnson, Baltimore, MD), filtered between 0.1 and 1 kHz and recorded on magnetic tape (TEAC 22-4 multitrack recorder with Vetter 2D FM adapter). An audio track was used to provide a vocal record, and on-line visual inspection of the recording was *via* a Tektronix 5112 oscilloscope.

After recording, tapes were reviewed and the first second (100 ms to 1100 ms) of the highest quality records was digitized. Only sensilla with complete stimulus series were included, and only one sensillum per animal was used to maximize the number of preparations. This resulted in a complete computer analysis of responses from one sensillum each from nine *L. decemlineata*, nine *L. haldemani* and 12 *L. texana*. The complexity of the records and the technique of computer analysis used (see below) made the above conditions necessary.

Spike separation and classification were made with the assistance of the SAPID Tools software package described in Smith *et al.* (1990) and used for the first time in their present form by Mitchell *et al.* (1990*b*). With this approach, waveform classifications made with the assistance of the computer on the basis of single recordings are initially scrutinized and edited by the operator by comparing all the records from each sensillum in turn. This results in a much more reproducible assignment of waveforms to spike classes within sensilla, since temporal variation in sensillar resistance etc. can be accounted for by comparing relative template shapes. Only after analysis of records from each sensillum independently were complete were results across sensilla considered. At the cross-sensillum comparison stage, very few editorial changes can be made to the classification due to inter-sensillum variability, but this step assists in final decisions regarding which waveform classes are likely to arise from homologous cells. Obviously, this approach only makes sense within homologous sensilla. Sample screens used in the analysis of a single record are shown in Fig. 1, and a brief description of a typical analysis is provided in the caption. Part of the screen used to compare several records from a sensillum or from homologous sensilla appears in Fig. 4. For most analyses included herein, template deviation was restricted to 15 % and deviation within a spike class to 20 %. Smith *et al.* (1990) give a more complete account of the use of SAPID Tools for this kind of analysis and the extensive cross-referencing involved. They also discuss the limitations of the method, one of which is that only records with a very good signal-to-noise ratio can be successfully analyzed.

Preparation and use of plant saps

Stimuli tested electrophysiologically were saps from *S. dulcamara*, *S. elaeagnifolium*, *S. tuberosum* var. Norland and *L. esculentum* var. Earliana; 100 mmol l^{-1} KCl was used as a control. Leaf saps were prepared by first grinding freshly cut leaves in liquid nitrogen with a mortar and pestle. 20 g of frozen leaf powder was then mixed with 40 ml of cold 100 mmol l^{-1} KCl and the resultant slurry centrifuged at 2000 *g* for 5 min. The supernatant was divided into 1.5 ml samples in

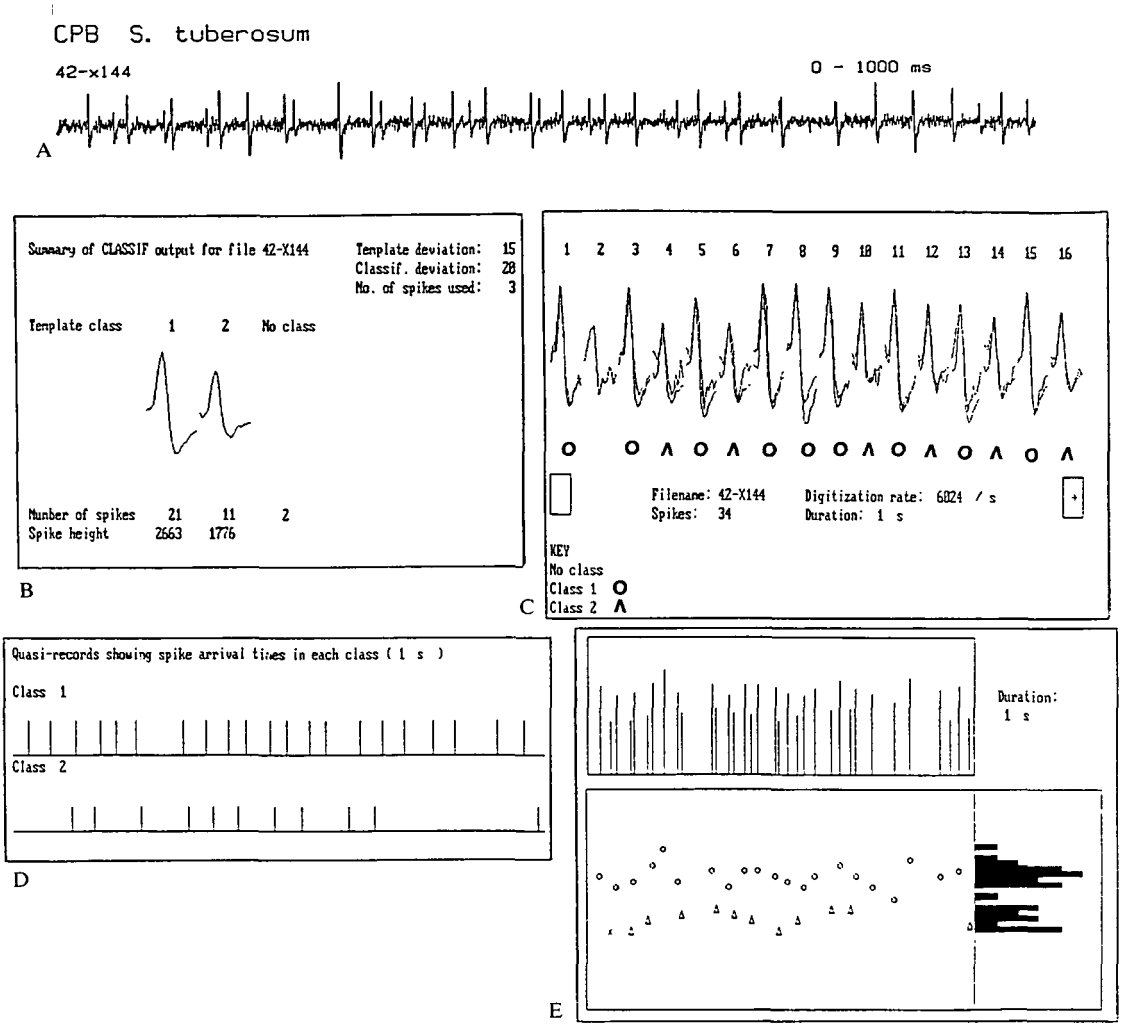

Fig. 1. Computer output screens (A–E) illustrating a typical analysis of a single record. Stimulus was potato homogenate. (A) Original response (100–1100 ms). (B) Result of classification with 20 % deviation allowed in assigning a waveform to a class (see also Fig. 4). (C) The first 16 waveforms each overlayed with its assigned template. This screen is used for visual determination of 'goodness of fit' for all waveform/template matches. Various template deviation and classification deviation parameters (screen B) can be tested until the best fit is obtained. (D) Arrival times for classified waveforms. This is used to confirm that spikes from a cell with a regular firing pattern have not been placed in different classes. (E) Reconstruction of original trace with only height and time of occurrence included, along with a height/frequency histogram. (Class 1, ○; Class 2, △). All of these screens are used to assess the quality of each classification result. Colour is used to provide much-improved resolution over what can be shown here.

Eppendorf tubes and immediately frozen in liquid nitrogen and stored on dry ice. Chlorophyll content of the supernatant was determined using the method of Bruinsma (1963) to guard against any gross differences in extraction efficiency among successive preparations of sap and as a general test for excessive oxidation or hydrolysis. Chlorophyll a and chlorophyll b ($mg\,l^{-1}$) were determined as well as chlorophyll a+b. Differences exceeding 5 % between the two determinations was taken to indicate excessive breakdown and the extract was discarded.

100 $mmol\,l^{-1}$ KCl was used to dilute plant saps to reduce plugging of the tip of the stimulating pipette by fine particles. Sensilla of *L. decemlineata* rarely respond to this concentration of KCl. *L. haldemani* and *L. texana* were slightly more sensitive to 100 $mmol\,l^{-1}$ KCl, so all preparations were tested and those that responded vigorously to this control solution were rejected.

Electrophysiological recording was confined to the third and seventh hours of photophase, which corresponded to the hours for which behavioural results were available. Sap from an acceptable plant was used to select a sensillum with a good signal-to-noise ratio response (*S. tuberosum* for *L. decemlineata*; *S. dulcamara* for *L. haldemani* and *S. elaeagnifolium* for *L. texana*). Once a sensillum had been selected, the second stimulus was 100 $mmol\,l^{-1}$ KCl, and the sensillum was rejected when the response to KCl was excessive. If the sensillum was acceptable according to the above criteria, the next two saps were applied using a random choice, excluding *L. esculentum*. *L. esculentum* was always the next to last stimulus used since bursting responses were often elicited by this sap several seconds into stimulation. Since this effect of *L. esculentum* may have indicated cell damage, and to determine reproducibility in general, the first sap of the series for that particular sensillum was re-tested. A disadaptation period of 3 min was allowed between the *Solanum* saps and one of 5 min following *L. esculentum* sap. All stimuli were applied for 15 s. Preliminary analysis revealed no differences in the relative response patterns for the first and fourth seconds, though absolute frequency declined with time. It is unlikely that the galeal sensilla remain in contact with leaf sap for more than 1–6 s at a time during normal feeding (B. K. Mitchell, personal video observations). However, sensory responses to plant saps are vigorous and adapt slowly, firing at 50 % of the original frequency after 9–10 s of continuous stimulation (Mitchell *et al.* 1990*a*). There may be other information in the later stages of the response, but this was not analyzed in the present study.

Results

Behaviour

Individual beetle–plant interactions were assessed using three of the component behaviours described by Harrison (1987): approach time, exploration time and feeding rate ($bites\,s^{-1}$).

Approach times (Fig. 2) were uniform within insect species, plant species having

no effect on walking speed. *L. texana* adults moved significantly more slowly than the other two species during this phase ($P<0.001$, d.f. 182 545).

Exploration times, time from initial contact until the first bite, are also given in Fig. 2. This behaviour also varied among insect species, with *L. texana* showing overall the longest bouts of exploration ($P<0.001$, d.f. 182 545). In addition, multiple comparisons using TukeyB indicated that *L. texana* explored leaves of its host plant *S. elaeagnifolium* for significantly shorter times than it did the other three plants. All other differences between individual exploration time means were non-significant.

The greatest differences, both across plants and across insects, occurred with feeding rate (bites in the first minute following the first bite). These results are presented with non-biters excluded (Table 1 and Fig. 2). Regardless of plant, beetle species showed major differences in feeding rate, with *L. texana* the slowest ($P<0.001$, d.f. 182 543). The feeding rate of each beetle species also depended on plant species.

For *L. decemlineata*, feeding rate was highest on *S. tuberosum* and *S. dulcamara*, with no difference between these two plants. There was also no difference in feeding rate by *L. decemlineata* on *S. elaeagnifolium* and *L. esculentum*, the least acceptable pair of plants. *L. haldemani* fed with equal rates on *S. tuberosum*, *S. dulcamara* and *L. esculentum*, only *S. elaeagnifolium* causing a large and significant reduction. *L. texana*, as expected from its monophagous habit, fed most rapidly on *S. elaeagnifolium*.

All but one of these trends remained when non-biting beetles were removed from consideration (Table 1), the exception being *L. texana* on *S. dulcamara*. The main advantage of removing the non-biting beetles is the apparent increase in feeding rate, particularly for *L. texana*. These values are a better reflection of actual feeding rate in the first minute by beetles that actually fed under the experimental conditions, especially in the short time used in the experimental protocol. Our general observations indicate that all beetles would eventually begin feeding, at least taking a single bite, given longer than the 3 min allowed in this assay.

Table 1. *Mean number of bites in 60 s (includes only beetles that fed)*

	Insect species		
Plant species	*Leptinotarsa decemlineata*	*Leptinotarsa haldemani*	*Leptinotarsa texana*
Solanum tuberosum	51±3 (50)	50±4 (33)	8±3 (5)
Solanum dulcamara	65±3 (50)	55±5 (26)	18±6 (6)
Solanum elaeagnifolium	34±3 (37)	11±1 (27)	27±5 (16)
Lycopersicon esculentum	39±4 (45)	41±5 (31)	5±1 (6)

Values are mean±S.D. (*N*).

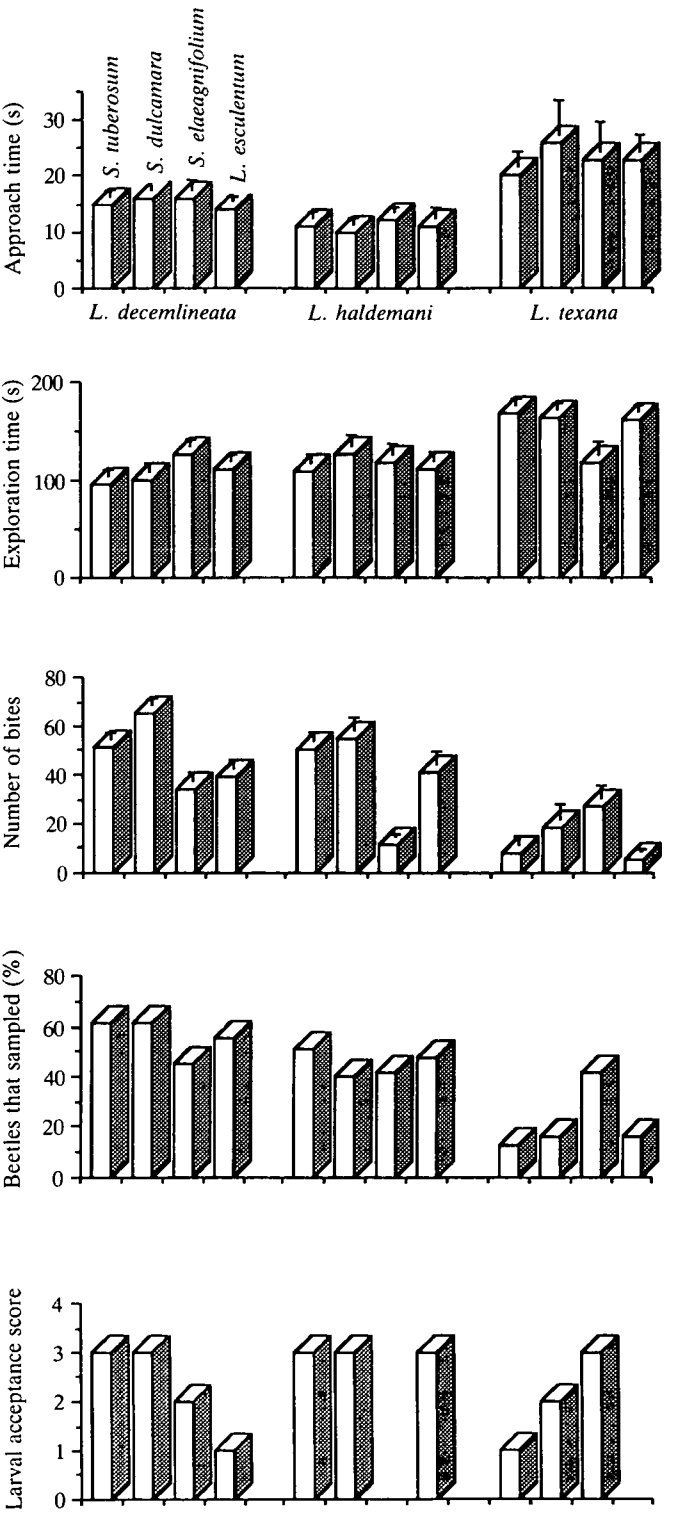
S. tuberosum
S. dulcamara
S. elaeagnifolium
L. esculentum
Approach time (s)
0
10
20
30
L. decemlineata
L. haldemani
L. texana
Exploration time (s)
0
100
200
Number of bites
0
20
40
60
80
Beetles that sampled (%)
0
20
40
60
80
Larval acceptance score
0
1
2
3
4

Fig. 2

Fig. 2. Summary of behavioural data for all beetle/plant species combinations. The four plant species are in the same order in all sections (see top left for order). N=82, 65 and 38 for *Leptinotarsa decemlineata*, *L. haldemani* and *L. texana*, respectively. The number of bites included only beetles that took at least one bite (no 0 scores), and Table 2 should be consulted for N values. Error bars are S.E. Larval data (bottom) are from Hsiao (1974), where 0 refers to rejection and 1, 2 and 3 refer to increasing acceptance (feeding/growth bioassay).

Fig. 2 summarizes the above results graphically and provides additional information on the percentage of beetles that sampled as well as on larval suitability of each of the experimental plants, taken from Hsiao (1974). From this it can be seen that most *L. texana* adults did not take a bite from offered leaves in the 3 min observation. Using the terminology of Harrison (1987) such beetles did not show gustatory sampling behaviour. Even on their host plant *S. elaeagnifolium*, these beetles had a low sampling rate, though it was significantly higher than for the non-hosts.

Sex of the beetle did not significantly affect any of the variables measured.

Sensory physiology

Leaf saps stimulated a number of cells in the galeal sensilla of the three beetle species, giving a variety of response patterns from virtually unicellular to complex patterns composed of responses from three or four cells. Variability was common, especially with regard to frequency of firing of modestly or poorly responding cells across stimuli. In certain insect–plant combinations (for example *L. decemlineata* and *L. esculentum*) even more general aspects of the response were not predictable. For example, responses to tomato varied from very weak to something resembling responses to potato (see Figs 4 and 5). With the above cautionary note in mind, sample individual records from one sensillum of an *L. texana* adult are presented in Fig. 3. With only single records such as these, there is no point in comparing the effects of the various saps by counting spikes and looking for differences of a few impulses per second. These records are shown only to indicate the type of responses obtained with the sap stimuli, and the quality of signal-to-noise ratio required for the analysis employed. The figure also shows how responses from one sensillum can vary in quality during an experiment. For instance, the response to *S. tuberosum* sap, in this particular series, showed the best signal-to-noise ratio of the four plant saps tested on this sensillum. Multicellular activity is present in all records. For meaningful analysis, a number of such records must be analyzed and the results averaged or viewed simultaneously before interpretable patterns begin to emerge.

Fig. 4 exemplifies the preceding point. Here responses of nine galeal sensilla taken from nine adult *L. decemlineata* to saps of *S. tuberosum* and *L. esculentum* are summarized. The waveforms represent computer-calculated templates of the spikes in each of four classes for each of the nine sensilla. For instance, with *S. tuberosum* sap, a cell with a large waveform consistently fired and is represented as

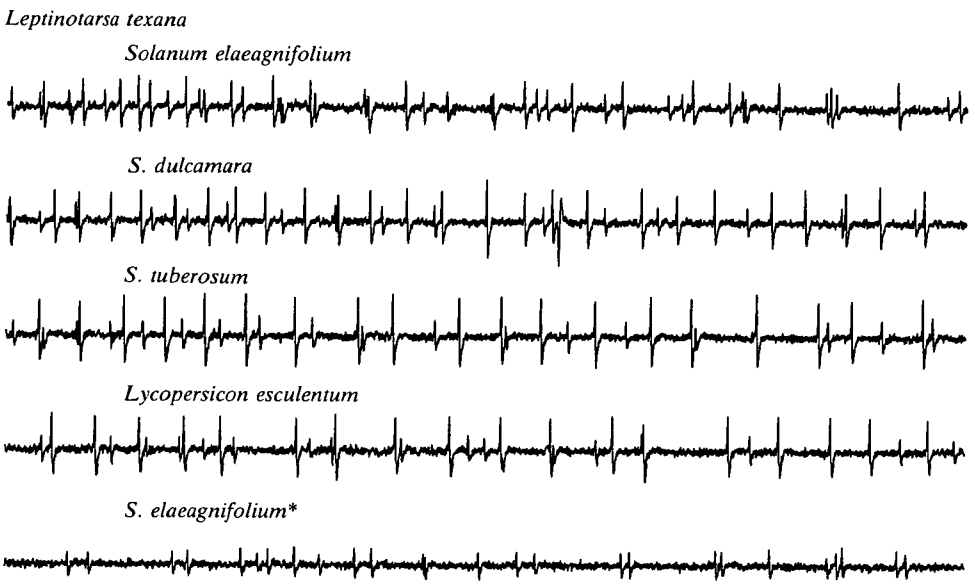

Fig. 3. Representative recordings from a galeal sensillum of *Leptinotarsa texana*. All traces are from 100 to 1100 ms. * The second response to *Solanum elaeagnifolium* is reduced since it followed a stimulation by *Lycopersicon esculentum* sap. See text and Fig. 6.

cell 1 (column 1 in Fig. 4). This same cell also fired in two of the sensilla when *L. esculentum* sap was the stimulus. Because of the similarity in the waveforms, as determined from several calculations and observations using SAPID Tools programs, we conclude that the same cell was involved in all nine responses to *S. tuberosum* and in the bottom two responses to *L. esculentum*. The same analytical procedure was used to make the other classifications indicated in Fig. 4.

Four cells are stimulated by both plant saps, but this is only consistently true for cell 1 with *S. tuberosum*. Clearly, the responses to *L. esculentum* are more variable than those to *S. tuberosum*, and cells 2–4 are more active with *L. esculentum* than with *S. tuberosum*. The same data are displayed in another manner in Fig. 5, with the addition of information on mean spike activity. Cells 1–4 in Fig. 5 are directly comparable to cells 1–4 in Fig. 4. In Fig. 5, the greater activity, with *L. esculentum* sap, from cells 2–4, and particularly from cell 3, is clearly illustrated. Also, the relatively unicellular response to *S. tuberosum* sap, suggested in Fig. 4, is further illustrated here. Cell 2 has weak activity (5.11 impulses s^{-1}), but this is well below the response of cell 1 (22.67 impulses s^{-1}). By contrast, *L. esculentum* sap stimulated all four cells, but note the extreme variability of these responses.

Analyses similar to the one detailed above were carried out for all insect/plant combinations, and the results are summarized in Fig. 6. Also, in discussing these results, we are less concerned with significant differences than with response patterns. The responses of *L. decemlineata* sensilla to *S. tuberosum* and *L. esculentum* sap discussed above are summarized in Fig. 6A.

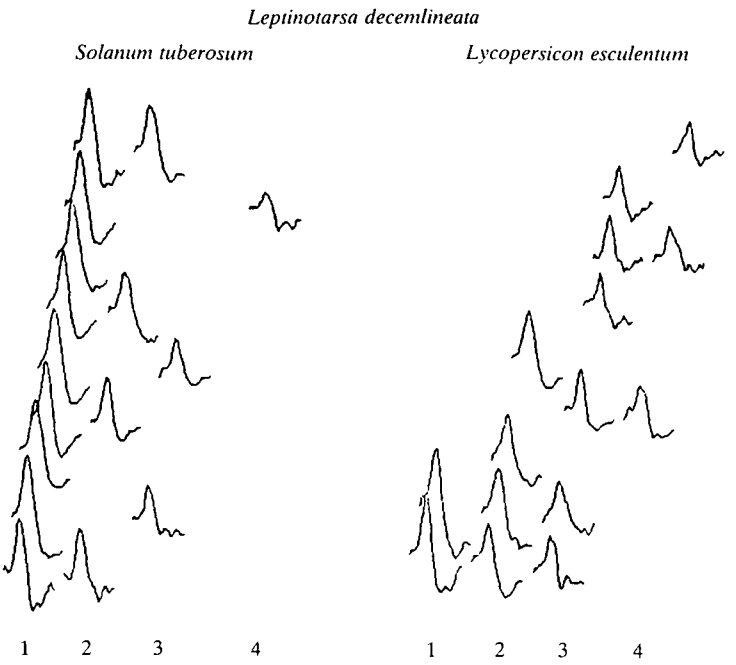

Fig. 4. Summary of analysis of responses from nine sensilla of *Leptinotarsa decemlineata* to saps of *Solanum tuberosum* and *Lycopersicon esculentum*. 1, 2, 3 and 4 on the bottom of the figure refer to cell types, as determined by the template-matching algorithm in SAPID Tools (Smith *et al.* 1990). Waveforms shown are for average templates representing each cell type and were generated by the computer.

Discussion

Behaviour

Although approach time distinguished *L. texana* from the other beetle species, this was not useful for measuring differences among plants within beetle species (Fig. 2). Because of this lack of variation in approach behaviour towards the plant species studied here, it is possible that these beetles have a limited capacity to distinguish among solanaceous plants at a distance of 10 cm. This result agrees with those of Visser and Nielsen (1977), who showed that starved adult female *L. decemlineata* increased upwind locomotion (decreased approach time) when the odour of solanaceous species was presented, even when the plant was a non-host such as *S. luteum* or *S. nigrum*. Most of the non-*Solanum* plants that beetles 'recognized' in this way in Visser and Nielsen's experiments were from solanaceous genera. They concluded that olfactory orientation in *L. decemlineata* would cause beetles to move selectively towards solanaceous hosts. Our results suggest that this may be true for *Leptinotarsa* species in general, especially since a monophagous species like *L. texana* might be expected to show olfactory discrimination among the three solanaceous 'non-hosts', one of which was

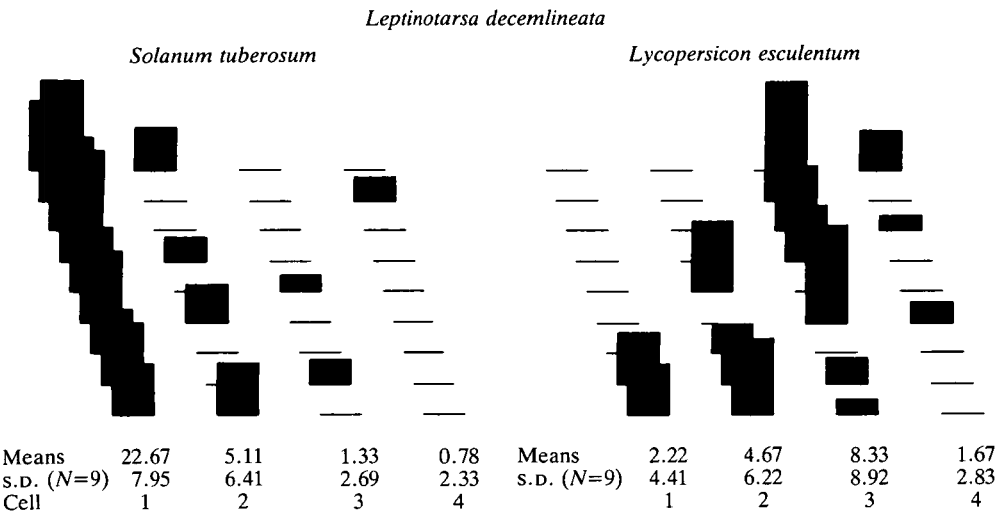

Fig. 5. Computer representation of the data illustrated in Fig. 4. Instead of plotting waveforms, this shows the relative magnitude of response (impulses s^{-1}) from each cell (1, 2, 3, 4). Means are impulses s^{-1} and the height of each histogram bar represents the response from a single cell. The nine sensilla used are ordered as in Fig. 4 (top to bottom). Cells 1, 2, 3 and 4 can be equated across the two stimuli (*Solanum tuberosum* and *Lycopersicon esculentum*).

subsequently eaten in the test. It would be interesting to test this hypothesis using a wider array of plants with *L. texana*.

Harrison (1987) described the behavioural category of 'examination' as including walking, palpating and antennal waving, while in contact with the plant. All of these active movements were seen in the three beetle species. The monophagous *L. texana* explored its host *S. elaeagnifolium* for a significantly shorter time than it did the other three plants. Even though there were no significant differences of this kind with *L. decemlineata* or *L. haldemani* (Fig. 2), there was a weak tendency for *L. decemlineata* to spend less time exploring *S. tuberosum*. Such a pattern of reduced exploration time and increased bites was demonstrated in a correlative analysis by Harrison (1987), who compared adult *L. decemlineata* from three locally host-adapted populations. Thus, when the host plant was offered, exploration time was lowest and feeding rate highest, even though these beetles had no opportunity, as adults, to learn the taste of their host. This strongly suggests some degree of innate chemosensory pattern-recognition mechanism which, when satisfied, allows a rapid decision to feed and normal feeding. This is particularly apparent in *L. texana*, the monophagous species, which completed exploration of its host within a mean time 30% shorter than that for the other plants.

Comparison of approach time and exploration time for *L. texana*, where host discrimination is absent on approach but clearly present after contact and before biting, suggests that odour concentration at the leaf surface is sufficient to allow

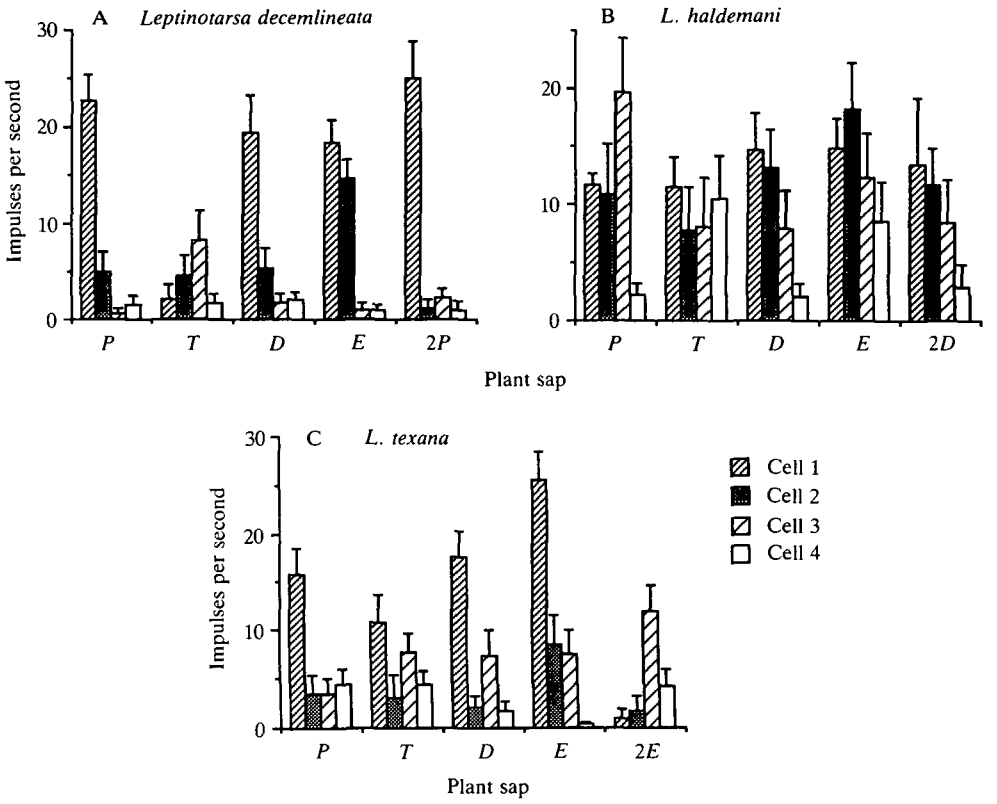

Fig. 6. Summary of data for all plant/insect combinations studied. Cells 1, 2, 3 and 4 can be equated with those shown in Figs 4 and 5. Impulses per second refers to the number of impulses between 100 and 1100 ms of the response. *P*, *Solanum tuberosum*; *T*, *Lycopersicon esculentum*; *D*, *S. dulcamara*; *E*, *S. elaeagnifolium*; 2*P*, 2*E*, 2*D*, second application in the series of *S. tuberosum*, *S. elaeagnifolium* and *S. dulcamara*, respectively. N=9 or 10 sensilla from as many animals for each species (see text).

discrimination of the host and non-host in this species. However, another explanation of this result is possible. *S. elaeagnifolium* leaves are heavily invested with non-glandular stellate trichomes. These give the leaf a silver sheen, and they create a different texture from those of the other plants which can probably be detected by the beetle immediately upon contact. Thus, in addition to the chemical pattern-recognition system discussed above, naive *L. texana* may also 'recognize', the unusual surface texture of their host, leading to shorter exploration times and a quicker onset of feeding. Interestingly, the trichome layer did not result in longer exploration times on *S. elaeagnifolium*, relative to other plants, for the other two insect species.

Schneider (1987) states that gustatory sampling is an important step in host plant discrimination. Indeed, the feeding rate, an approximation of gustatory sampling, allowed ranking of plant species acceptability for the three beetle species. For *L.*

decemlineata, *S. tuberosum* and *S. dulcamara* are more acceptable than the other two species tested; For *L. haldemani*, these two species are equally acceptable, but *S. elaeagnifolium* was less acceptable than the other three; and for *L. texana*, the natural host plant *S. elaeagnifolium* was more acceptable than the other three plants. Interestingly, for *L. texana*, the graduation in acceptability across plants evident in larval assays was not seen in adult bioassays (Fig. 2).

When feeding rate is calculated after excluding those beetles that did not bite, the above ranking of host acceptability holds, with the exception that *S. dulcamara* apparently increases in acceptability relative to the other plants. Possibly for *L. texana* gustatory sampling of *S. dulcamara* provides similar stimuli to *S. elaeagnifolium*. If a beetle proceeds to the stage of gustatory sampling, *S. dulcamara* becomes nearly as acceptable as the host plant. This supports the idea that leaf texture could be an important element for host recognition by *L. texana*, since, when the trichome mat is absent, very few beetles will bite into even a chemically suitable leaf within 3 min.

In conclusion, the behavioural assays show that actual contact with the leaf contents in the form of initial biting provides essential information for host plant recognition by *Leptinotarsa* beetles. Using the measure of bites in the first minute of feeding, plant acceptability rankings can be made that are consistent with measures of plant suitability for larval growth and field observations of plant association.

Sensory physiology

Mitchell *et al.* (1990*a*) argued that, in cases where a single taste cell fired reliably and frequently while other cells fired weakly and unpredictably, then the majority, if not all, of the information must be carried by the most reliable input. This type of situation was demonstrated for *S. tuberosum* sap and *L. decemlineata*, and the basic result is confirmed here. The advantage of the present data is that the analysis is based on a much improved version of the computer software. Figs 5 and 6 show these results, and the large difference in coefficient of variance (CV) between the activity of cell 1 and that of the other three cells can be calculated from the values given in Fig. 5. CV for cell 1 is 29% and for the next most consistent cell, cell 2, it is 120%. In addition, the activity of cell 1 completely overshadows the activity of the other three cells in the response to potato sap (Fig. 6). In comparing the histograms shown in Fig. 6, it is important to keep the above point in mind. Much of the low-level activity (below 6 impulses s^{-1}) may not contribute at all to the neural code that enables the insect to recognize its host plant.

Of course, variability can be high, even with mean activity greater than 6 impulses s^{-1}, which is the case with cell 3 of *L. decemlineata* responding to *L. esculentum* sap (Fig. 5). It is possible that generally erratic responses, such as those of *L. decemlineata* sensilla to *L. esculentum*, generate a kind of code that is interpreted as 'non-host', 'novel' or 'foreign', and that leads to limited feeding. Mitchell *et al.* (1990*a*) also found that tomato sap elicited such an erratic response,

Table 2. *Coefficients of variation for means greater than 6 impulses* s^{-1} *from Fig. 5*

	Cell 1	Cell 2	Cell 3	Cell 4
Leptinotarsa decemlineata				
Solanum tuberosum	29	–	–	–
Lycopersicon esculentum	–	–	88	–
Solanum dulcamara	49	–	–	–
Solanum elaeagnifolium	32	29	–	–
Leptinotarsa haldemani				
Solanum tuberosum	22	98	59	–
Lycopersicon esculentum	56	118	132	86
Solanum dulcamara	56	64	98	–
Solanum elaeagnifolium	108	56	78	93
Leptinotarsa texana				
Solanum tuberosum	42	–	–	–
Lycopersicon esculentum	66	–	64	–
Solanum dulcamara	37	–	93	–
Solanum elaeagnifolium	29	91	86	–

– means 6 or less.

and the reader is referred to that paper for further discussion on the implications of such inputs and possible mechanisms leading to such sensitivity.

Though high variability could simply signal 'foreign', one would expect additional coding mechanisms to exist to allow finer distinction between, for instance, *Solanum* species. These more precise codes would certainly have to be more reliable than the highly variable 'foreign' code; in fact, degree of variability could be one measure that will help distinguish those parts of a complex response pattern that comprise a finer-grained code. Implicit in this approach is the assumption that not all of the sensory cell activity present in a response to a plant sap has equal weight in fine-grade coding and that some of this activity, in fact, may be entirely noise.

The coefficient of variation (CV) is a measure of variability in which variance is expressed relative to the magnitude of the response (S.D. as a percentage of the mean). If we assume that lower CVs correspond to greater information-carrying capacity of the sort necessary to be useful in fine-grade codes, then an upper limit CV must exist above which little or no useful information can be extracted. Correspondingly, the best possible signal for a fine-grade code would be one with perfect fidelity, a CV of 0%. Table 2 gives the CVs for all of the means plotted in Fig. 6 that are greater than 6. Means of 6 or less had CVs greater than 100% with one exception of 95%. Since it is not known how high a CV must be before it is useless as part of a fine-grade code, or if this 'usefulness' measure varies linearly with CV, we must, for the present argument, make a somewhat arbitrary choice of cut-off. From these data, and data on *Sarcophaga bullata* (Mitchell *et al.* 1990*b*), it is likely that CVs of 20–50% are normal for insect taste systems, even when all of

the sensilla are taken from the same preparation. Thus, we assume that responses with CVs up to 50 % will contribute to fine-grade coding. CVs from 50 to 100 % are probably less important in this respect, and we will assume that responses with CVs above 100 % contribute nothing.

In the CV comparison shown in Table 2, the most reliable responses from cell 1 were to *S. tuberosum* sap by *L. decemlineata* sensilla (CV 29 %), to *S. elaeagnifolium* sap by *L. texana* (CV 29 %) and to *S. tuberosum* sap by *L. haldemani* (CV 22 %). Cell 1 of *L. decemlineata* was also fairly consistent in its response to *S. elaeagnifolium* (CV 32 %). Broadening the criteria to include CVs up to 50 %, for cell 1 in *L. decemlineata* and *L. texana*, only *L. esculentum* failed as a reliable stimulus. By the same criterion, only *S. tuberosum* was a reliable stimulus for cell 1 in *L. haldemani*, though saps of *L. esculentum* and *S. dulcamara* had CVs of 56 %. For cell 2, only that of *L. decemlineata* in response to *S. elaeagnifolium* was in the under 50 % category, and no responses of cells 3 or 4 were under 50 % for any insect.

This suggests that cell 1 is extremely important in host recognition, at least in *L. decemlineata* and *L. texana*. For *L. decemlineata*, the low frequency (Fig. 6) and high variability of cell 1 responses to *L. esculentum* probably function primarily as a coarse-grained code and contribute to the low acceptability of this plant. Acceptability of *S. elaeagnifolium* by *L. decemlineata* was also low, despite the reliable input from cell 1. However, the highly reliable input from cell 2 may be sufficient to allow the insect to distinguish between this plant and its host plant. Histograms of impulse frequencies for cells 1 and 2 elicited by *S. tuberosum* and *S. elaeagnifolium* illustrate this (Fig. 6).

L. texana sensilla responded poorly to *L. esculentum* (Fig. 6), even though the response of cell 1 was fairly reliable (CV 66 %). However, the low-frequency response of cell 1 and the fairly high and erratic response of cell 3 are probably sufficient to make this plant unacceptable. In addition, *L. texana* sensilla were very sensitive to long-term effects of *L. esculentum* sap (Fig. 6). The final stimulation in each experiment was always a repeat of the host sap. In *L. texana*, there was a clear reduction in sensitivity of cell 1 to *S. elaeagnifolium* sap 5 min following stimulation with *L. esculentum* sap. We did not systematically measure other possible effects of tomato sap, but this sap can cause bursting responses in some sensilla following prolonged application (Haley, 1988). These were similar to the effects reported for solanine, tomatine and chaconine on the same system by Mitchell and Harrison (1985), though not as extreme. *L. texana* may be particularly sensitive to components of *L. esculentum* sap, perhaps alkaloids, which further disrupt the already variable physiological response to this plant. This effect was not seen with *L. decemlineata* (Fig. 6).

L. texana also fed poorly on *S. tuberosum* (Fig. 2), yet the response from the galeal sensilla to *S. tuberosum* sap was similar to that of the host plant, *S. elaeagnifolium* (Fig. 6 and Table 2), with the exception of the higher response of cell 1 to host sap. *S. dulcamara*, a reasonably acceptable plant to those *L. texana* beetles that bit into it, had a response profile very similar to that of *S. tuberosum*.

Thus, the small difference between responses to *S. elaeagnifolium* and *S. tuberosum* (higher cell 1 activity to host sap) cannot be the cause of the clear difference in acceptability between the plants. Other sensilla with different specificities may allow the beetles to distinguish between *S. dulcamara* and *S. tuberosum*, even though the galeal input is identical.

The data for *L. haldemani* are the most difficult to interpret. The recordings obtained in this study varied in quality, with sensilla from *L. texana* routinely producing superb signal-to-noise ratios and spike patterns of reasonable complexity. Records from *L. haldemani* were the poorest, though the nine chosen were within the limits required by the analysis used. *L. decemlineata* sensilla yielded records that ranged quite widely, but some were as clear as those from *L. texana*. Often responses from *L. decemlineata* to *S. tuberosum* and *S. dulcamara* were practically unicellular. Our confidence in the analysis clearly varies with the quality of the records, and it is lowest for *L. haldemani*. Nevertheless, qualitative differences between *L. haldemani* and *L. texana* or *L. decemlineata* are clearly evident (Fig. 6 and Table 2). More cells did respond at higher frequencies in *L. haldemani* sensilla (Fig. 6) and the responses were more variable (Table 2). However, this variability makes it difficult to compare any of the means plotted on the histograms in any detail. One possibility is the higher response of cell 2 to the unacceptable plant, which was fairly consistent (CV 56%). However, since this cell was active with all saps, it seems a weak candidate to explain such a dramatic behavioural difference. Interestingly, the high response of cell 3 to potato with a CV of 59% was not associated with a change in acceptance of this plant. The active responses of three of the four cells in *L. haldemani* sensilla to all saps could be related to its wider taxonomic range of acceptable plants. Conversely, in *L. decemlineata* and *L. texana* in particular, we may be seeing a more finely tuned group of cells that allow more precise host recognition.

The sensory results discussed here were from a single type of sensillum in each of 9–12 beetles; thus, the variability referred to is between animals. Clearly, a single beetle does not have access to the chemosensory input of its neighbour, so the variability of greatest importance to the above hypotheses is inter-sensillar variability within single animals. To approach this directly would require a completely different experimental design to the one we used. Also, the requirement of very good signal-to-noise ratio recordings would make obtaining suitable data, from say 10 sensilla per beetle, tedious in the extreme. Nevertheless, such an experiment is required to test the hypotheses presented above, perhaps with a smaller number of plants, to reduce logistical problems and analysis overload, and attempting to get at least six good sensilla per animal. Mitchell *et al.* (1990*a*) compared inter-sensillar and inter-animal variation in *L. decemlineata* and found that 64% of cell 1 variance was due to inter-animal variance when *S. tuberosum* sap was used as the stimulus. However, activity of the other cells varied widely over sensilla both within and among animals. There was even considerable variation in activity, other than in cell 1, among successive stimulations of the same sensillum. Such differences may yield important information when multiple plant

and insect species are compared with repeated stimuli and multiple sensilla per animal. The observation that cell 1 does vary less within animals (Mitchell *et al.* 1990*a*) suggests that the conclusions reached here regarding this cell would be even easier to demonstrate within animals.

Clearly the three beetle species differ in the sensitivity of their galeal sensilla to complex stimuli, namely saps of acceptable and non-acceptable plants. There is no simple set of sensory patterns that correlates with acceptable or unacceptable, though perhaps none should be expected. The results support the hypothesis that highly variable input could be a coarse-grained code signalling 'foreign' and hence unacceptable. The case of *L. haldemani* suggests that more-polyphagous species relay less-precise sensory information and that the central nervous system allows a high degree of variability in 'recognised' patterns. In more oligophagous or monophagous species, the sensory system appears to be more highly tuned (less variable), and comparison of input patterns of several cells by the central nervous system may constitute part of a fine-grained code distinguishing among chemically more similar acceptable and unacceptable plants.

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