Electrophysiological Responses of the L1 and Lateral Styloconic Sensilla Sugar-

Sensitive Cells of the Spruce Budworm *Choristoneura fumiferana* (Clem.) in

Response to Various Carbohydrates

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ABSTRACT

Electrophysiological Responses of the L1 and Lateral Styloconic Sensilla Sugar-Sensitive Cells of the Spruce Budworm *Choristoneura fumiferana* (Clem.) in Response to Various Carbohydrates

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Spruce budworm larvae have 2 sugar-sensitive cells, one in the lateral styloconic sensillum (LST) of the galea and one in the L1 sensillum of the maxillary palp, indicating the importance of sugars to these insects. Several sugars, such as sucrose and fructose, are important phagostimulants. In fleshflies, two sites are responsible for binding with sugars, a pyranose (P) site that binds pyranose sugars (i.e. glucose), and a furanose (F) site that binds furanose sugars (i.e. fructose). The LST of spruce budworms responds to both pyranose and furanose sugars. In the present study it was found that, unlike the LST, the L1 sensillum in sixth instar larvae does not respond to any of the pyranose sugars tested. Furthermore, the L1 response to sucrose and fructose differed from that of the LST. This indicates that the two sugar-sensitive cells may have differences in the sites (P- and F-) that they possess. The K_b and V_{max} of the L1 sensillum demonstrated a high affinity for fructose; the threshold of this sensillum was also lower to fructose than the threshold of the LST to this sugar. The response to fructose for both sensilla reaches a plateau somewhere beyond 500 mmol/L. The characteristics of the responses from both the L1 and LST agree with previous behavioural studies regarding the spruce budworm's preference for sugars, and also correlate well with the physiological data regarding the

sugars present in the host-plants of this insect. The role of the L1 in relation to the LST and in budworm feeding behaviour is discussed. The hypothesized role of the L1 is to detect water stress in plants, and that of the LST is believed to be the detection of sugars as a token stimulus. Since the physiological characteristics of the two sugar-sensitive cells present in both these sensilla correspond well with behavioural data as well as the sugar concentrations found in spruce budworm host plants, both the L1 and LST are likely to play an important role in feeding behaviour.

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Introduction

The interactions between lepidopterans (butterflies and moths) and their host-plants are better understood then those of other insects (Bernays, 1992). The order Lepidoptera consists of about 10 percent of all animal species (Schoonhoven and Van Loon, 2002). Lepidopterans are generally considered very discriminating eaters, and the majority of them are specialists, i.e. they feed only on a few plant species compared to generalists, which feed on many plant species (Schoonhoven and Van Loon, 2002). Caterpillars are considered ideal animals for the study of food selection behaviour because of their small size, amenability, and relatively simple sense of taste (Schoonhoven, 1977; Schoonhoven and Van Loon, 2002). Given the myriad of chemicals present within plants, it is surprising that an animal with such a relatively simple sensory system is capable of sensing such a diverse array of compounds (Bernays, 1992; 2001).

In order to comprehend an insect's feeding behaviour, efforts must first be made to understand its chemosensory code, which is the underlying sensory input that forms the basis of that feeding behaviour (Mitchell, 1979; Schoonhoven and Van Loon, 2002). Behaviour is an expression of neural processes which are modified by physiological as well as environmental factors, and an understanding of the constraints and functioning of the nervous system is integral in understanding feeding behaviour (Bernays, 2001), and in developing efficient management strategies for pests, such as the spruce budworm.

The spruce budworm *Choristoneura fumiferana* Clem. (Lepidoptera: Tortricidae) is a major defoliator of coniferous forests in eastern North America; it is an oligophagous insect that feeds primarily on balsam fir *Abies balsamea* (L.) Mill., white spruce *Picea glauca* (Moench) Voss., red spruce *Picea rubens* Sarg., and black spruce *Picea mariana* Mill. (Miller, 1975). The spruce budworm maintains a low population profile for periods of 30 to 80 years before erupting to outbreak proportions (Miller, 1975). Outbreaks occur at irregular intervals (Blais, 1973), but the average period between outbreaks is approximately 35 years (Kimmins, 1971). These outbreaks, dating as far back as the 1700's, are a part of the natural ecological cycle of forest maturation and succession (Baskerville, 1975; Kucera and Orr, 1981).

The spruce budworm is of major economical interest to the pulp and paper industry, owing to the fact that it is a major defoliator of softwood species (Miller and Rusnock, 1993). Defoliation of current year foliage of balsam fir causes a reduction in growth of both stem and roots (Piene and Little, 1990). After one or more years, heavily defoliated trees die (Kucera and Orr, 1981). While both young and old trees are susceptible to attack, it is the older, mature trees that are more vulnerable and suffer the greatest amount of defoliation (Miller, 1975). Budworm outbreaks kill vast stands of spruce-fir forests; the estimated damage caused by this insect during the last century is believed to be well over 450 million cords (1 cord is equal to 128 cubic feet or 3.624 556 meters) in eastern North America (Blais, 1973).

The spruce budworm has one generation per year. From mid to late July, female moths search for suitable host trees on which to lay their eggs. These pale green eggs are deposited in long row-like masses usually on the underside of the fir or spruce needles (Miller, 1975; McGugan, 1954). After approximately ten days the eggs hatch as first instar larvae, only about 2 mm long, which immediately disperse into the spruce/fir canopy in search for a place to spin their overwintering shelters, or hibernacula (Kucera and Orr, 1981; Miller, 1975). The caterpillars molt to the second instar and spin hibernacula which last throughout winter, during which the larvae remain dormant in diapause until spring. In late April to early May the second instars emerge from diapause to begin feeding (Miller, 1975). The newly emerged second instars mine the preceding year's needles, as well as staminate cones or buds (McGugan, 1954). The larvae go through four more molts and reach the sixth instar in mid June. The sixth instar has the longest duration (McGugan, 1954), and is the larval stadium that causes the greatest amount of feeding damage (Miller, 1975).

In late June the larvae stop feeding and pupate, emerging as adult moths approximately ten days later (Kucera and Orr, 1981). Males mature faster then females, so the first moths to emerge from the puparia are males (McGugan, 1954). The virgin females that emerge are unable to take flight; they mate within a day then deposit about 20 eggs in several egg masses on the same tree on which they matured (Miller, 1975). After mating and oviposition takes place, usually by the second day, the females are capable of active flight. Peak flight occurs from 7:30 p.m. to around 11:30 p.m. Moths can cover great distances, and can reach as far as 90 km (Miller, 1975; Kucera and Orr,

1981). When they disperse the females will lay the rest of their egg complement, for a total of about 200 eggs.

The survival of the spruce budworm is closely related to the phenology of its host trees (Quiring and McKinnon, 1999). Spruce budworms normally feed on current year foliage after budbreak (Miller, 1975; Blais, 1979). According to feeding preferences of the spruce budworm for polar compounds extracted from host trees, white spruce should be the most preferred of its host species (Albert and Parisella, 1988a), but they preferentially defoliate balsam fir because of its early budbreak (Albert and Jerrett, 1981). Black spruce suffers less defoliation due to its late budbreak (Mattson et al., 1991; Blais, 1957), and its late phenology is also why its cones receive greater damage than its foliage (Prévost and Laing, 1986). Asynchrony between the budworm and its host trees results in increased mortality to the insect (Eidt and Cameron, 1971).

Outbreaks are not only associated with the maturing of large areas of host trees, especially flowering balsam fir, but with climatic variation as well. Spring and autumn droughts as well as three or four consecutive dry summers are conditions that favor budworm outbreaks (Hardy et al., 1983; Blais, 1973). Sugars and amino acids, which are important elements of osmoregulation in many plants, increase in stressed plants, as is the case with black spruce (Zwiazek and Blake, 1990), and carbohydrates are the most stimulating of the water-soluble host-plant chemicals to spruce budworms (Albert and Jerrett, 1981; Albert and Parisella, 1988a). High temperatures are also known to increase

feeding rates of sixth instar larvae (Retnakaran, 1983) as well as budworm developmental and survival rates (Mattson et al., 1991).

The increase in staminate flowers in times of stress, as seen during hot dry weather (Greenbank, 1963), could also be responsible for increased budworm survival. Kimmins (1971) noted that flowering fir trees have higher concentrations of amino acids and nitrogen in their foliage than non-flowering balsam fir trees, which are important to younger instars (Albert and Bauce, 1994; Harvey, 1974). More flower buds, which open prior to vegetative buds, may allow more post-diapause second instars to establish feeding sites and grow to maturity (Mattson et al., 1991). Mattson et al. (1991) has shown that there is a higher survival rate for young larvae in flowering branches versus non-flowering branches, and suggested that the increased survival of spruce budworms is due to the increased availability of shelter provided by the host tree's flower buds, and not due to the host's nutritional status. However, the authors also mention that naturally occurring drought-stress could result in increased levels of sugars, nitrogen, and other nutrients important to insect, thereby resulting in higher levels of budworm survival and fecundity.

Foliage age also influences insect development and survival. For example, the foliage of young (30-year-old) trees present at the time when the young (pre-sixth instar) spruce budworm larvae are feeding possesses more nitrogen, has a higher nitrogen:tannins ratio, and less total soluble sugars then foliage from older (70-year-old) trees (Bauce et al., 1994). A high nitrogen:tannins ratio means that there are more

proteins available because they are not bound to tannins, which are some of the plants defensive compounds. Bauce et al. (1994) found that budworms reared on mature 70-year-old trees caused a greater amount of defoliation, had lower mortality, and shorter development times than those that were reared on juvenile (30-year-old) trees. Furthermore, larvae that were reared on foliage from young trees and then transferred to foliage from old trees after they reached the sixth instar had shorter development times than those that were not transferred. So while greater tree age has a negative effect on young instars, it has a positive effect on older, sixth instars. During the time of actively feeding sixth instars (i.e. late June) the foliage in young trees contains more nitrogen, free sucrose, free glucose, free fructose, and total soluble sugars, but it also contains more secondary compounds than foliage in old trees. The high levels of monoterpenes in young trees throughout this period, which reduce the ingestion rate of sixth instar larvae, coupled with the fact that the younger foliage also has a lower nitrogen:tannins ratio (Bauce et al., 1994) could be the reasons that sixth instars fare better on foliage from older trees than that from younger ones.

Sugars, particularly sucrose, are common in all plants as the products of photosynthesis (Schoonhoven et al., 1998) and are primary constituents of the host-plants of the spruce budworm (Neish, 1958; Harvey, 1974; Little, 1970). Nutritional studies done by Harvey (1974) have shown the significance of several sugars in spruce budworm development, while studies performed by Albert et al. (1982) indicated the preference of this insect for particular sugars, especially sucrose.

Sucrose is a powerful phagostimulant for spruce budworms (Albert et al., 1982), as it is for all lepidopterans, indicated by the presence of a sugar-sensitive cell in all species tested (Schoonhoven, 1972; Chapman, 2003). The detection of sugar may be particularly important for sixth instars, where higher levels of sugar are needed for the final molt into pupae (Panzuto and Albert, 1997).

The sense of taste, or gustation, may be the most important sense involved in the selection of food (Mitchell, 1979). The balance between cues that induce feeding (phagostimulants) and those that deter feeding (phagodeterrents), along with their integration within the insect brain, is what drives feeding behaviour (Chapman, 2003; Schoonhoven, 1987). The sense organs, or sensilla, that are responsible for perceiving these cues also act as filters, relaying information of only certain environmental conditions to the brain, and therefore play a role in the decision process (Schoonhoven, 1987). Insects have the capacity to discriminate non-host-plants from host-plants by comparing the total inputs from their various sensilla (Schoonhoven and Dethier, 1966). Sensilla are capable of perceiving different chemicals such as nutrients as well as token stimuli, such as secondary plant compounds (Schoonhoven and Dethier, 1966). In caterpillars, the main sensilla involved in gustation are those present on the maxillae. Each maxilla bears two relatively large peg-like sensilla termed the medial (MST) and lateral (LST) styloconic sensilla. The tip of each maxillary palp bears a group of much smaller sensilla, three of which are presumed to be gustatory on the basis of earlier work done in our lab (Albert, 2003). The labrum's inner surface or epipharynx also bears two small dome-like sensilla on each side, which are known to be gustatory in other

caterpillars (Schoonhoven and Van Loon, 2002), as well as in the spruce budworm (Albert, 1980). The LST and MST are the sense organs believed to be the most important in mediating feeding behaviour (De Boer, 1991; Schoonhoven, 1972). However, there is evidence that the maxillary palps, which may be used alternatively to taste and smell in small larvae (Schoonhoven, 1972), may also play an important role in taste discrimination (Blom, 1978), but individual palp sensilla have not yet been studied in any caterpillar except the spruce budworm (Albert, 2003).

Insects can perceive chemicals present on plant surfaces using their maxillary palps. Epicuticular waxes present on the surface of plant leaves contain chemicals which insects can detect by drumming their palps on the surface (Blaney and Duckett, 1975). Thus, palpatation of the leaf exterior enables the insect to determine whether the leaf is a suitable food source before ever having to take a bite (Schoonhoven, 1987; Bernays and Lewis, 1986; Woodhead and Chapman, 1986). Furthermore, testing the surface of a plant before biting circumvents the possibility of ingesting toxic compounds, even in small amounts, that may be present within the leaf (Woodhead and Chapman, 1986). Following palpatation the insect will respond by either taking a bite of the leaf, and may continue feeding if the internal constituents of the leaf are also palatable, or the insect will reject the leaf and search for a more suitable food source upon which to feed (Schoonhoven et al., 1998). Chapman (1976) has suggested that it was also advantageous for plants to develop chemicals on the outer surface of their leaves advertising the chemical content within in order to avoid unnecessary tissue damage. Thus recognition of an unsuitable host-plant prior to biting is of value to both the insect as well as the plant.

Sensilla have different morphologies that correlate with their function. For example, sensilla used in smell have many small openings in their cuticle (multiporous), whereas sensilla used in taste have a single pore opening at their tip (uniporous) (Chapman, 2003). Dendrites of chemosensory neurons that are responsible for conveying information directly to the central nervous system, extend into the tip of the sensilla (Bernays, 1992). In gustation, plant chemicals enter through the pore at the tip upon contact of the sensillum with the substrate, and interact with the receptors present on the dendritic membranes of the innervating neurons (Mitchell, 1979; Chapman, 2003). So when maxillary palps come into contact with chemicals, like the dry, non-polar compounds contained in epicuticular waxes, the chemicals bind with the dendritic receptors present within the sensillum and the information is sent to the brain as action potentials (Bernays, 1992). The type of stimulus and its strength is encoded in the action potential by its amplitude, frequency, and adaptation rates (Bernays, 1992; Schoonhoven and Van Loon, 2002). Aside from the temporal patterns of the action potential, the brain of the insect can integrate the information in two other ways, via labeled-lines or across-fiber patterns. In the labeled-line coding mechanism the insect brain can make a decision on whether or not to feed based on information from a single neuron without requiring input from additional neurons. In across-fiber patterns, the information from various neurons is assessed and a decision is made based on the input from the two or more neurons (Bernays, 1992; Schoonhoven et al., 1998). Simmonds and Blaney (1990) noted that the labeled-lined system might just be one extreme of across-fiber patterning. Furthermore, Schoonhoven et al. (1998) suggested that in interpreting complex chemicals, such as plant saps, the across-fiber pattern is employed. However, when

dealing with certain compounds, such as deterrents, single cells exert a more dominant role and thus the labeled-line system would be used. Each gustatory cell has a sensitivity spectrum, but the chemical that evokes the strongest response is usually considered the best stimulus for that cell (Schoonhoven, 1987). Moreover, while the spectra between cells differ they may also overlap (Schoonhoven and Dethier, 1966).

There are many factors that influence receptor specificity, such as larval stadium, within-instar age, time of day, nutritional status, and past experience (Schoonhoven and Van Loon, 2002). Receptors can also undergo changes in number or in resting potential/threshold during the insect's life (Blaney et al., 1986; Panzuto and Albert, 1997). The changes that occur at the peripheral level influence the insect's preferences during the course of its life, as does feedback on the receptors from a centrally regulated mechanism such as direct or indirect action on receptors by hormones, or the nutrient content of the haemolymph (Blaney et al., 1986; Chapman, 1999). Blaney et al. (1986) also suggest that relatively small changes in the sensitivity of receptors can cause significant behavioural modifications. For example, a caterpillar could change its sensitivity to certain aspects of its host plant if it has become used to this plant during the course of its growth. This is the case with the tobacco hornworm, which prefers solanaceous foliage when reared on plants from the family Solanaceae, rejecting suitable non-solanaceous foliage, but remains polyphagous when reared on non-solanaceous food. This has been attributed to modification of the caterpillar's taste receptors that results in the receptors being tuned to certain host-chemical recognition cues (del Campo and Miles, 2003). The significance of changes that can occur at the receptor level is that the

peripheral changes are involved in the decision process, which is therefore not confined to the brain of the insect alone. This makes for better use of the nervous system, as well as a reducing the work of the CNS (Blaney et al., 1986).

By decoding gustatory codes we can gain a better knowledge of the important aspects of how an insect recognizes a potential food source (Schoonhoven, 1968), and use this information in developing better pest management strategies. The prime objective of these strategies, which generally consist of aerial insecticide applications, biological control strategies, and forest management (Blais, 1957; Kucera and Orr, 1981), is to minimize the defoliation caused by insect pests (Miller, 1976). Therefore a good understanding of an insect's life cycle, phenology, and especially feeding behaviour and its underlying mechanisms is needed in order to employ effective pest management methods (Régnière, 1982; Dethier, 1969; Schoonhoven, 1968). This can be accomplished through electrophysiological studies of the sense organs present on the mouthparts of the insect in conjunction with behavioural tests (Schoonhoven et al., 1998; Albert, 1980; Panzuto and Albert, 1997).

Few electrophysiological studies have been done on caterpillars; the studies to date have focused mainly on the LST and MST of such caterpillars as the tobacco hornworm *Manduca sexta* (De Boer et al., 1977), the silkworm *Bombyx mori* (Ishkawa, 1967), the white cabbage butterfly *Pieris brassicae* (Ma, 1972; Blom, 1978), the cabbage moth *Mamestra brassicae* (Blom, 1978), and the spruce budworm *Choristoneura fumiferana* Clem. (Lepidoptera: Tortricidae).

Electrophysiological responses have been shown to be closely correlated to feeding behaviour in the spruce budworm, especially with regards to the LST of sixth instars and their feeding response to sugars (Panzuto and Albert, 1997; Albert et al., 1982). The LST present on the galea of the maxillae has been shown to be an important sensillum in mediating the feeding behaviour of lepidopterans (De Boer 1991; Schoonhoven, 1972). Numerous ablation experiments have shown that removal of this sensillum in the oligophagous caterpillar *Pieris brassicae* (Blom, 1978; Ma, 1972) resulted in a decrease in food discrimination. Bilateral removal of LST and its adjacent sensillum, the MST, along with the epipharyngeal sensilla, results in the greatest loss of food discrimination, indicating that in *Pieris brassicae* the input of LST, as well as MST and epipharyngeal sensilla, is essential for proper food discrimination (Blom, 1978).

The LST of the spruce budworm contains 5 neurons, one of which responds to sugars (Albert, 1980). The physiological responses of this cell were shown to be closely correlated with behavioural responses, further indicating the importance of this sensillum in feeding behaviour (Albert and Parisella, 1988*b*).

Ablation of the maxillary palp of various lepidopterans, like *Manduca sexta* (Glendinning et al., 1998) and *Pieris brassicae,* also resulted in less discriminative feeding behaviour, suggesting that it too plays a significant role in feeding (Blom, 1978). Glendinning et al. (1998) indicated that olfactory and taste receptor inputs from the maxillary palps in the tobacco hornworm (*Manduca sexta*) were sufficient, but not

necessary, for host-plant responses. From these experiments the maxillary palp seems to play a significant, but more generalized role in feeding than the LST.

The maxillary palp of the spruce budworm has eight basiconic sensilla of which only three have been identified as gustatory (Albert, 1980). One of these, sensillum L1, has been shown by Albert (2003) to contain a sugar-sensitive cell as well as a water-sensitive cell. Aside from Albert (2003), no other electrophysiological studies are available for this sensillum for any lepidopteran species.

It is surprising that the spruce budworm possesses two sugar-sensitive cells, given the limited number of taste cells present in caterpillars (Schoonhoven and Van Loon, 2002; Schoonhoven, 1977). The characteristics of the sugar-sensitive cell in L1 differ, however, from those of the LST. According to Albert (2003), LST has a lower threshold and plateau than L1 and thus a greater sensitivity to sucrose.

The fact that there are two sugar cells in two different sensilla on the maxilla, and that their characteristics are different, suggests that they might have different functions. The role of LST has been hypothesized to be the detection of sugar as a token stimulus, due to the fact that its low sensitivity and plateau correspond to the levels of sucrose found in host-plants (Albert, 2003). Detection of water stress in plants is thought to be the role of the L1 sensillum, since in times of drought the level of some sugars can increase to well over 50 mmol/L in stressed plants, such as black spruce (Zwiazek and Blake, 1990); levels which the LST would be unable to detect.

A sugar-cell is believed to have multiple sugar receptor sites which according to Shimada et al. (1974), who conducted his experiments on flies, includes pyranose sites (P-sites) and separate furanose sites (F-sites). Additionally there seems to be sites that bind aromatic amino acids (Ar or Aryl sites) as well as sites that bind aliphatic carboxylates (R or Alkyl sites) in the sugar-sensitive neuron of flies (Shimada, 1987).

Regarding sugars, glucose, in the α-glucopyranose form, binds to the P-site while fructose, in the fructofuranose form, binds to the F-site. Since sucrose dissociates completely, 100% of it is available to bind to the receptor. Only 33% of glucose, however, is in the α-glucopyranose form and available to interact with the P-sites. Similarly, only 20% of fructose is in the fructofuranose form and available for interaction with the F-sites. In flies sucrose is believed to stimulate the P-sites and F-sites almost equally, and the sum of the responses from the individual receptor sites is equal to the response to sucrose (Cheung and Smith, 1998).

From preliminary studies conducted on L1 by Albert (unpublished data), the fructose and sucrose responses are similar but the glucose responses are low. This may mean that, assuming the sugar-receptor of the spruce budworm behaves in a similar way to the one found in flies, there may be no P-sites present.

The LST of spruce budworms has been shown to respond to both pyranose and furanose sugars, with both fructose and glucose eliciting responses from the sugar-sensitive cell (Panzuto and Albert, 1997). If the sugar cell in the LST is similar to that

present in flies, this would suggest the presence of P- and F-sites within the LST sugar-sensitive cell. One of the most important furanose sugars to the budworm is fructose, while glucose is a highly utilizable pyranose sugar (Harvey, 1974). In light of the differences in response to sucrose between the LST and the L1 sensilla (Albert, 2003), it stands to reason that there may exist differences in their response to fructose and glucose as well, given their effect on budworm growth and development (Harvey, 1974).

The goals of this thesis were to ascertain the response characteristics of the L1 sugar-sensitive cell to various sugars and compare them to the response characteristics of the LST sensillum. Analyzing the differences between the two sugar-sensitive cells should yield valuable information on the role of these cells, as well as the sensilla they occupy, in spruce budworm feeding behaviour.

Materials and Methods

Insects

Sixth-instar larvae were used for all experiments. The insects were obtained as second-instar post diapause larvae from the Forest Pest Management Institute in Sault Ste Marie, Ontario, and were reared on artificial diet.

Diet was made according to Grisdale (1988), with the exception that only 1.2 grams of Aureomycin (5.5% active) was initially incorporated into the diet instead of 20 grams that was incorporated after experiment 6, because the 1.2 grams was originally believed to be a sufficient amount of antibacterial agent. Cheesecloth-parafilm strips containing the second-instar larvae were cut into small pieces and placed into 23 ml plastic cups filled with about 10-15 ml of the diet. The cups were then closed off with cardboard lids and placed upside-down on a paper-lined tray in an incubator. The temperature was set at 22°C with 60% humidity and a photoperiod of L16:D8, and insects were kept under these conditions for a period of about two weeks. The insects were then transferred to Petri dishes containing diet. The diet, incubators, and any other materials used for rearing, except the insects themselves, were periodically irradiated with a Spectroline 120 volt shortwave Ultraviolet 254 nm light, model XX-15G (Spectronics Corporation, Westbury, NY, USA), for a period of 10 minutes to eliminate any pathogens that might be present.

Electrophyisology

Electrophysiological experiments were performed as in Albert (2003). Larvae were severed between the third thoracic segment and the abdomen; the head and the three thoracic segments were then mounted on a blunted glass micropipette. The pipette was filled with insect Ringer solution (Schnuch, 1990) to ensure that the insect remained alive during experimentation. Since the tip of the pipette extended into the hypopharynx it prevented the insect from moving and it also caused the extension of its galea and palps, thereby facilitating experimentation. The insect preparation was then placed, with the ventral side up, on a reference electrode and observed with a Leitz Laborlux 12 compound microscope. The stage of the microscope had been removed and the preparation was viewed with a L32/0.40 objective using Periplan 10x/18 oculars. A Dolan-Jenner illuminator (Model 170D) was used for lighting. One branch of the split fiber optic cable was placed at an angle so that the light shone down on the insect preparation from above. The other branch provided illumination via the microscope's own lighting pathway.

Another glass micropipette containing the test solution was placed on the recording electrode and viewed under the microscope. During experimentation the tip of the test solution was touched to the tip of the sensillum using micromanipulators. The ensuing nerve impulses, or action potentials, were then recorded. Each stimulation lasted about one second and a three-minute rest period was given between each trial so that the sensory cells would not adapt. Only one palp of the insect was used for each experiment

unless otherwise stated. Except for experiments 7 and 8, a sucrose solution was always tested prior to experimentation in order to ensure that the insect preparation was alive.

Responses were obtained using a high impedance amplifier (10^{15} Ohms) attached to the recording electrode. A second amplifier was used to amplify the signals further, which were then recorded on digital audio tapes using a Sony 57ES digital audio recorder. All recordings were obtained as unfiltered DC signals to prevent any distortion associated with filtering. Sapid Tools (Smith et al., 1990) were used to digitize the recordings at a rate of 10,000 sample points per second and only the first second of the response was used. Recorded traces were printed on paper and the nerve impulses during the first second were counted and subsequently analyzed.

Solutions

All chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA). KCl in distilled water was used as the solvent in all solutions, at various concentrations, to ensure adequate conductivity, and solutions were always presented in random order except where otherwise mentioned.

Experiments

All insects were treated in the same manner except in experiment 6. Several controls were employed in experiment 6 in order to eliminate any confounding variables such as: within instar age (time from last molt, time until next molt), nutritional status (hunger level), sex (male, female), and within insect variability (left palp versus right

palp), that might have been influencing the results. For experiment 6, fifth instar larvae were placed into separate numbered Petri dishes (100 mm x 15 mm) containing diet. The Petri dishes were examined 3 times per day (morning, noon, and evening) to check for any newly molted sixth instar larvae. Upon molting to the sixth-instar each insect was placed into its own small, numbered, diet-containing Petri dish (32 mm x 12 mm). To control for within-instar age, the time that the sixth-instar was discovered as well as the time when the Petri dishes containing the fifth instar larvae were checked, and time of experimentation, were noted. From these data it was possible to obtain the approximate age of the insect by first calculating the minimum and maximum ages. The minimum age was calculated by measuring the time between the discovery of the sixth instar and the time at which the experimentation took place. Since the time of the discovery of the sixth-instar does not necessarily mean that the insect molted at that exact moment, a maximum age was also obtained. The maximum age was calculated by measuring the duration between the last time the fifth instar larvae were checked (prior to the discovery of the sixth-instar) and the experimentation time. Ages were calculated in hours and minutes. The maximum mean age of a male was 168 hours; the maximum age of a female was 240 hours. These values correspond to the duration of time it takes for males and females to complete the sixth stadium, that being 7 and 10 days respectively (Mulye and Gordon, 1990). The hours were rounded to the nearest day, so that an insect with a mean age of 80 hours and 29 minutes would be 3.3 days old, therefore between 3 and 4 days old.

All sixth instar larvae were starved for 24 hours prior to experimentation to control for nutritional status/hunger level, and the sex of the insect was also noted. For insects that had both palps intact and functioning, both palps were tested to see if there was any difference in response between them. Sucrose was tested at the beginning and end of each experiment to ensure that the insect was alive, and to see if there was any deterioration in response towards the end of the experiment. The rest of the solutions were tested in random order, and the order in which the solutions were tested was also recorded. Since no effects of starvation, palp, sex, or age were found, the rest of the experiments (7-10) were performed without these controls (as in experiments 1-5).

Statistical Analyses

All statistical analyses were performed using NCSS (J.L. Hintze, 865 East North, Kaysville, UT, USA). Descriptive statistics were used to obtain the mean and standard error of the responses to the solutions for all experiments. Various *t*-tests and ANOVAs were used to compare means within and between experiments, the specifics of which are described in the results.

Results

Experiment 1: L1 Sensillum Response to Various Fructose Concentrations

According to preliminary studies (Albert, 2003) the L1 sensillum's sugar-sensitive cell is very sensitive to D-fructose, which suggests that it possesses a furanose site (F-site) as described for flies by Shimada (1987). In the present experiment, five solutions in concentrations of 5, 10, 100, 200, and 500 mmol/L of D-fructose dissolved in 10 mmol/L KCl were tested in order to ascertain the response of the L1 sensillum to fructose.

D-fructose elicited a characteristic monophasic (a single peak of electrical current), positive sugar cell response (Fig. 1) as described in Albert (2003). It should be noted that at such a low concentration some of the spikes are difficult to count visually during the first 100 ms of stimulation, due to the difference in voltage (offset) between the recording electrode and the insect preparation. This is reflected in the high standard error (4.42) relative to the response for the threshold concentration of fructose (5 mmol/L).

Insect 3, Sensillum L1, 5 mmol/L D-Fructose
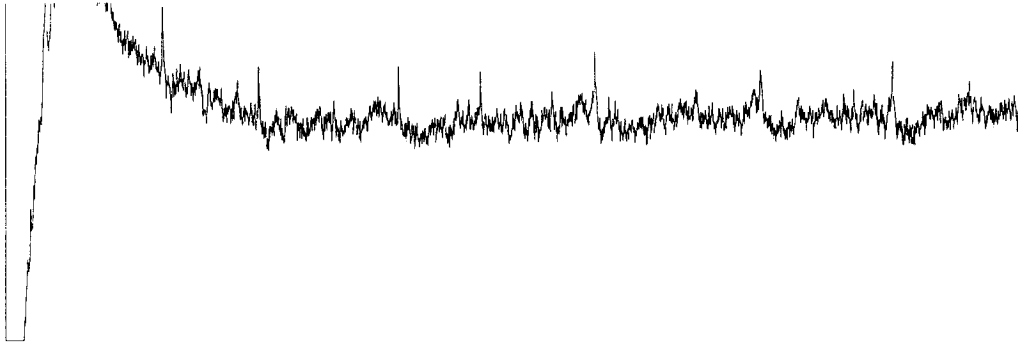
Insect 3, Sensillum L1, 100 mmol/L D-Fructose
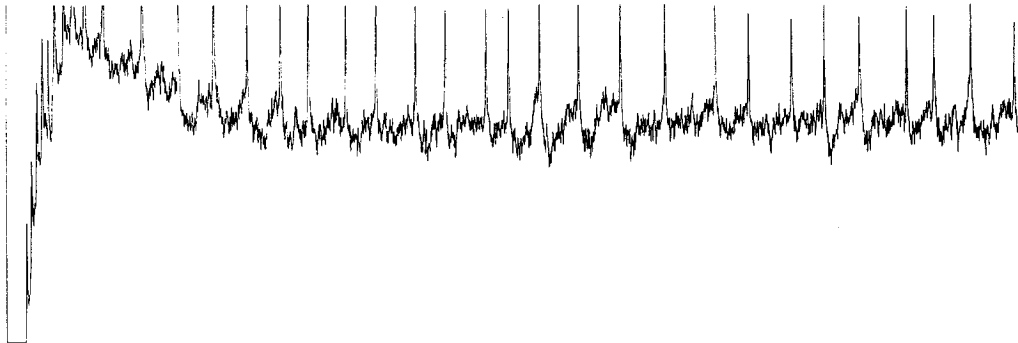
Insect 3, Sensillum L1, 500 mmol/L D-Fructose
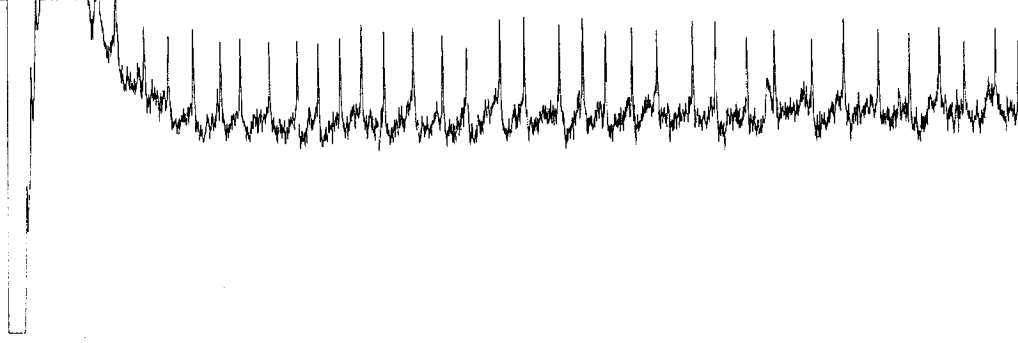
0	200	400	600	800	1000

Fig. 1: Electrophysiological traces of the L1 sensillum sugar-sensitive cell's response to concentrations of D-fructose dissolved in 10mmol/L KCl. Time bar = 1 second (or 1000 ms).

An ANOVA was used to determine if fructose concentration had an effect on response (mean impulses/s). A Bonferroni multiple comparison test was also utilized to determine differences among stimuli in order to ascertain the concentration at which the L1 sugar-sensitive cell response to fructose reaches a plateau of firing frequency.

As the concentration of D-fructose increases, so does the mean number of impulses/s. According to the one-way ANOVA (df = 4, 92; F = 14.86; $p < 0.001$) there is an effect of stimulus concentration on response. Moreover, a Bonferroni multiple comparisons test shows that the 5 mmol/L concentration differs from all other concentrations. It was less clear if the differences between the 50, 100, 200, and 500 mmol/L concentrations were significant or not. When all the data are plotted in a scatter plot and fitted with a log curve, it shows that the mean impulses/s still increase, though at a much lower rate (Fig. 2). Furthermore, a paired t-test (p < 0.05) performed between the 50 and 100 mmol/L concentrations ($t = -3.24$, $n = 19$, $p = 0.004$), the 100 and 200 mmol/L concentrations ($t = -3.91$, $n = 19$, $p = 0.001$), as well as the 200 and 500 mmol/L concentrations ($t = -3.83$, $n = 18$, $p = 0.001$), revealed a significant difference between all of them. Therefore the response plateau is not reached somewhere between the 50 and 100 mmol/L concentrations, but somewhere beyond the 500 mmol/L concentration.
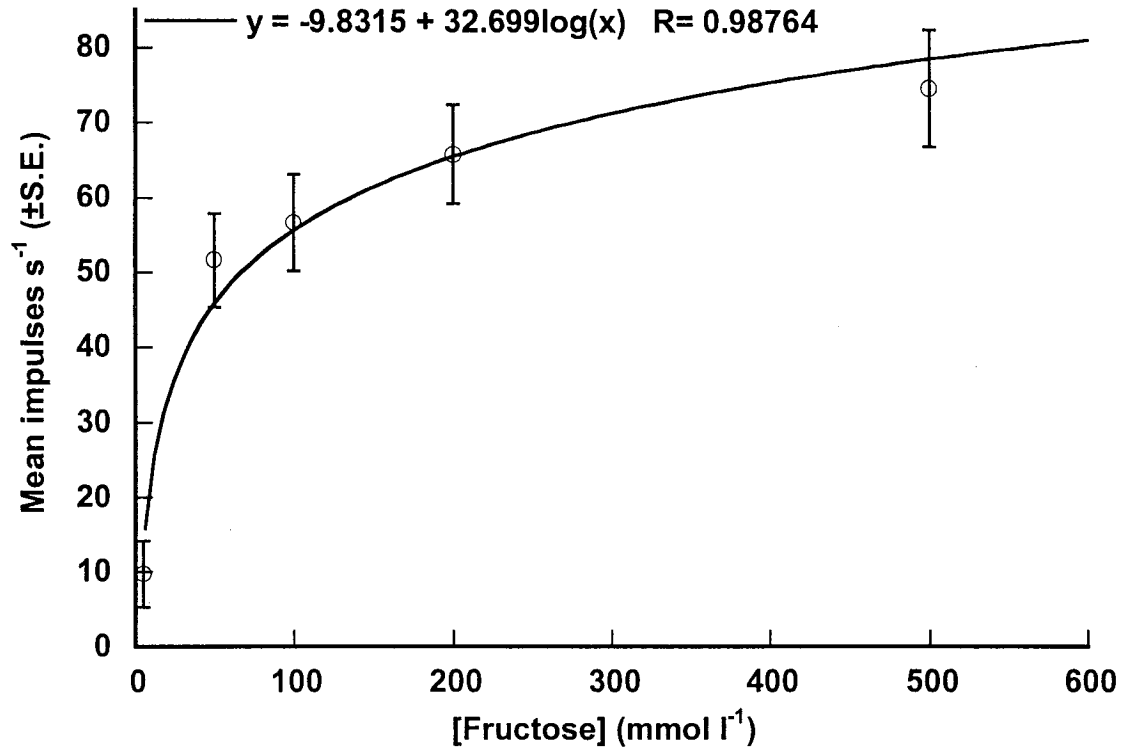
Fig. 2: Responses of sensillum L1 to D-fructose concentrations (±S.E.), n = 19 for each concentration except for the 5 and 500 mmol/L concentrations, where n = 18. Log curve = solid line.

To obtain the K_b, which is the concentration at which half of the maximal response occurs, and the V_{max}, which is the maximum response of the cell, of the L1 sensillum's response to fructose, the reciprocal of the concentrations used (i.e. 1/concentration) and that of the mean impulse/s (1/response) were plotted using a double-reciprocal Lineweaver-Burk plot (Lehninger, 1975). A regression line was fitted to the graph, which was then extrapolated in order to obtain the x- and y-intercepts (Fig. 3). The double-reciprocal Lineweaver-Burk plot is a transformation of the Michaelis-Menton equation. The y-intercept of the double-reciprocal plot is equal to $1/V_{max}$, and the x-intercept is equal to $-1/K_b$. By rearranging these equations, the V_{max} and the K_b can be

obtained. The Michaelis-Menton equation is normally used for enzymatic reactions, however receptor-substrate reactions are believed to be similar enough to enzyme-substrate reactions that the equation can be used for receptors as well. The V_{max} and K_b values of the L1 response to D-fructose for various time periods are listed in Table 1. However, since only 20% of fructose is in the fructofuranose form and able to stimulate the F-site in a sugar cell (Cheung and Smith, 1998), the adjusted K_b values (the V_{max} doesn't change) corresponding to 20% of the represented fructose concentrations used are also given. These values were obtained form graphs like Fig. 3.
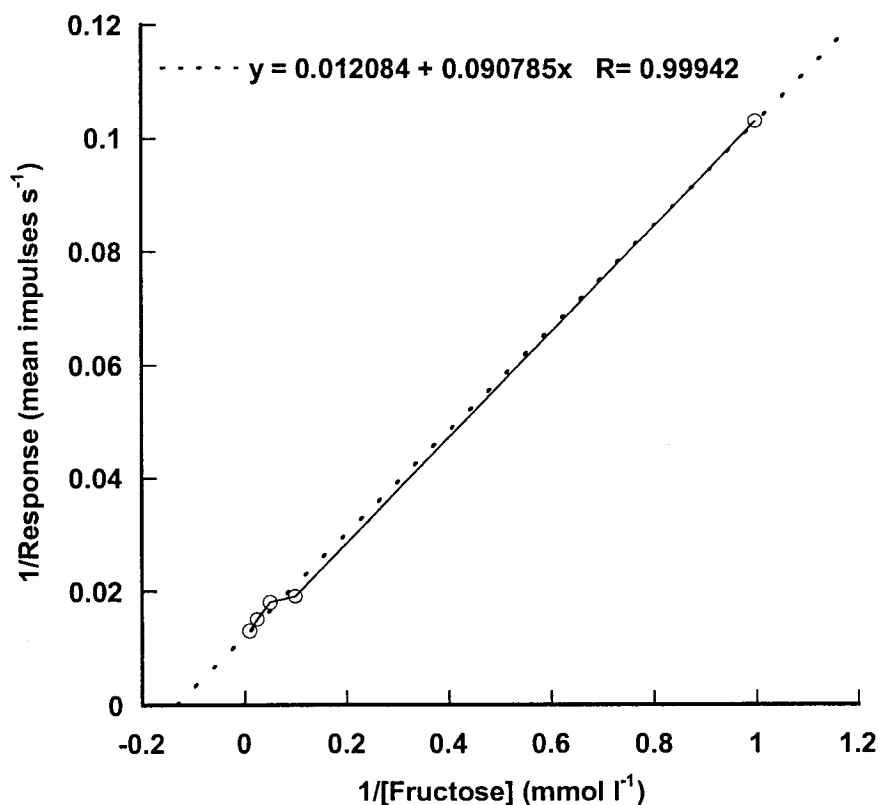
Fig. 3: Double-reciprocal (Lineweaver-Burk) plot of response of the L1 sugar-sensitive cell to fructose vs. 20% of the concentration of D-fructose. Dotted line = fitted regression line.

Table 1: Values of V_{max} and K_b from Michaelis-Menton double-reciprocal (Lineweaver-Burk) plots of response of the L1 sugar-sensitive cell vs. the given concentrations and the adjusted concentrations (20%) of D-fructose for time periods of 100, 200, 300, 400, 500, and 1000 ms.

Duration (ms)	Equation	V_{max} (impulses^{-s})	K_b (mmol^{-L})	K_b (mmol^{-L}) Adjusted	Equation Adjusted
100	$Y = 0.006X + 0.27$	175.4	48.1	9.5	$Y = 0.006X + 0.05$
200	$Y = 0.007X + 0.32$	135.1	44.0	8.7	$Y = 0.007X + 0.06$
300	$Y = 0.008X + 0.33$	117.6	39.1	7.8	$Y = 0.008X + 0.07$
400	$Y = 0.009X + 0.36$	107.5	38.2	7.7	$Y = 0.009X + 0.07$
500	$Y = 0.010X + 0.38$	100	37.9	7.6	$Y = 0.010X + 0.07$
1000	$Y = 0.45X + 0.012$	83.2	37.7	7.6	$Y = 0.012X + 0.09$

Experiment 2: L1 Sensillum Response to Various Glucose Concentrations

Pyranose receptor sites (or P-sites) are required for sensing pyranose sugars in flies (Shimada, 1987). The LST sensillum is sensitive to several pyranose sugars (Panzuto and Albert, 1997), one of which is glucose, which has a significant effect on spruce budworm development (Harvey, 1974). This indicates that the LST possesses P-sites (as well as F-sites). In order to determine if the L1 responds to glucose, solutions of 5, 10, 100, 200, and 500 mmol/L concentrations of D-glucose, with 10 mmol/L KCl as the solvent, were utilized. In our experiments with the L1 sensillum, however, no responses were obtained from any of the insects tested ($n = 6$) to any of the wide range of concentrations of D-glucose used.

Experiment 3: L1 Sensillum Response to Other Pyranose Sugars

While the L1 sensillum may not respond to glucose, this does not mean that it does not respond to any of the other pyranose sugars. Additional tests were performed with sugars that were found by Shimada (1987) to interact with the P-sites in flies (see Table 2). If the L1 possessed P-sites, like the LST, then it should respond to at least one, or some, of the other pyranose sugars being tested.

With the exception of sucrose, D-xylose, and 1-0-methyl-α-D-glucopyranoside, none of the sugars evoked any response from the L1 sensillum. Furthermore, the responses evoked by D-xylose and 1-0-methyl-α-D-glucopyranoside seemed more characteristic of water-sensitive cells (small, monophasic, positive spikes) than sugar-sensitive cells (large, monophasic, positive spikes).

Table 2: Mean impulses/s (±S.E.) of L1 sugar-sensitive and water-sensitive cells for 100 mmol/L sugar concentrations with a 10 mmol/L KCl solvent; n = 5. *Insects can respond to more than one sugar.

Number of Insects Responding to Sugar*	Stimulus (100 mmol/L)	Mean Sugar Cell Response (±S.E.)	Mean Water Cell Response (±S.E.)	# Insects Responding to water
5	Sucrose	54.2 (6.8)	0	0
0	D-xylose	0	3.6 (2.5)	2
0	1-0-methyl-α-D-glucopyranoside	0	1.8	1
0	Myo-inositol	0	0	0
0	Melezitose	0	0	0
0	L-arabinose	0	0	0
0	Raffinose	0	0	0

Experiment 4: L1 Sensillum Response to Pyranose Sugars at Lower Concentrations

The lack of a sugar cell response to the pyranose sugars in the previous experiment could have been due to an osmotic effect of the high sugar content (100 mmol/L) of the test solutions, which might have drawn the water out from the sensillum's terminal pore resulting in the lack of a response. In order to eliminate this as a possible effect, the concentration of the sugars, shown in Table 3, were lowered to 25 mmol/L.

Spikes characteristic of a sugar-sensitive cell response were observed for all these same sugars at the 25 mmol/L concentration, except melezitose and L-arabinose. However, only a small number of insects responded to the pyranose sugars (4 in total), while all insects responded to sucrose. Some insects responded to more than one pyranose sugar. Responses characteristic of a water-sensitive cell were seen for melezitose, as well as for myo-inositol (Fig. 4) and 1-0-methyl-α-D-glucopyranoside.

Insect 11, Sensillum L1, 25 mmol/L Myo-Inositol
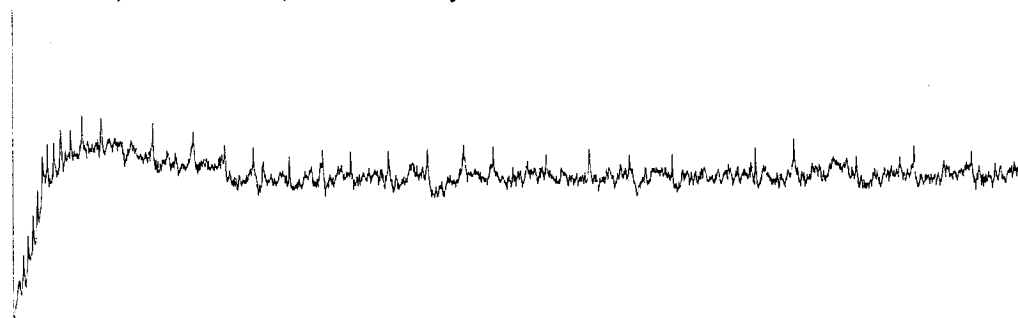
Fig. 4: Electrophysiological trace of the L1 sensillum to 25 mmol/L pyranose sugars with 10 mmol/L KCl solvent showing the response characteristics of a water-sensitive cell (cell responds to water and not myo-inositol).

Table 3: Mean impulses/s (±S.E.) of sugar-sensitive and water-sensitive cells for 25 mmol/L sugar concentrations with a 10 mmol/L KCl solvent; n = 13. *Insects can respond to more than one sugar.

Number of Insects Responding to Sugar*	Stimulus (25 mmol/L)	Mean Sugar Cell Response (±S.E.)	Mean Water Cell Response (±S.E.)	# Insects Responding to water
13	Sucrose	43.3 (4.3)	0	0
3	D-xylose	9.2 (5.5)	0	0
1	1-0-methyl-α-D-glucopyranoside	2.1	2.5	1
1	Myo-inositol	2.4	2.4	1
0	Melezitose	0	2.3	1
0	L-arabinose	0	0	0
4	Raffinose	11 (21.6)	0	0

Experiment 5: L1 Sensillum Response to Pyranose Sugars with a Higher Solvent Concentration

Increasing the salt concentration from 10 to 25 mmol/L of the solutions tested (Table 4) might increase the signal to noise ratio of the responses to sugar stimuli, due to an increased conductivity of the solution (Morita et al., 1967). A solvent concentration of 10 mmol/L was originally used because this concentrations usually provides adequate conductivity in most cases. In fact, a greater number of impulses/s from the sugar-sensitive cell was seen for almost all the sugars except for D-xylose and raffinose (Table 3). Only melezitose and raffinose gave responses similar to those of water-sensitive cells.

Table 4: Mean impulses/s (±S.E.) of sugar-sensitive and water-sensitive cells for 25 mmol/L sugar concentrations with a 25 mmol/L KCl solvent; n = 5. *Insects can respond to more than one sugar.

Number of Insects Responding to Sugar*	Stimulus (25 mmol/L)	Mean Sugar Cell Response (±S.E.)	Mean Water Cell Response (±S.E.)	# Insects Responding to water
5	Sucrose	47.4 (7.8)	0	0
2	D-xylose	8.4 (5.4)	0	0
1	1-0-methyl-α-D-glucopyranoside	5.6	0	0
1	Myo-inositol	4.0	0	0
1	Melezitose	5.0	2.4	1
1	L-arabinose	5.6	0	0
0	Raffinose	0	3.6	1
5	D-fructose	39 (7.0)	0	0

Analysis of the results revealed a high standard error (S.E.) for all sugars, except sucrose and D-fructose. The number of insects responding with the sugar-sensitive cell to each sugar, other than sucrose and D-fructose, was low, with only a small portion of insects (2 insects in total) responding to the pyranose sugars. This suggested that there was a high degree of variability in the population in the responses of the L1 sugar-sensitive cell to pyranose sugars; some insects seemed to possess the ability to respond to these sugars while the majority did not.

Experiment 6: Proportion of Insects Responding to D-xylose and Raffinose with the L1 sensillum

The pyranose sugars that gave the highest overall response in the previous experiments (25 mmol/L D-xylose and raffinose in 25 mmol/L KCl) were further tested

in order to obtain a better indication of the proportion of insects that can detect pyranose sugars with the L1 sensillum.

Several controls were used in case the variability observed in the L1 response to pyranose sugars was due to factors other than the possession of P-sites by a small number of insects. These included within-instar age, within-insect variability, sex of the insect, and nutritional status of the animal. It is also possible that the insect's response could have deteriorated throughout the experiment, since the insect is in the process of dying from the onset of experimentation (Gothilf and Hansen, 1994). This is unlikely, however, since all experiments were completed within 1 hour and therefore should not have resulted in any significant deterioration in response (Gothilf and Hansen, 1994). Nevertheless, a control for this was still performed by testing sucrose at the beginning and at the end of each test series.

The sex, palp, stimulus, order of solutions, and age were all assigned integer values to facilitate statistical analysis. For example, the right palp was assigned a value of 1 and the left palp a value of 2; male was equal to 1 and female was equal to 2. The integers assigned to the solutions were equal to the number of solutions tested and were always kept constant throughout the experiment (i.e. sucrose was always stimulus 1). Order 1 corresponded to solutions tested in the following order: sucrose, xylose, raffinose, sucrose, while Order 2 corresponded to: sucrose, raffinose, xylose, sucrose. Values of 1-10 were given for the age of insects according to approximately how old the insect was. For example, an insect between 3 and 4 days old would be given a value of 4,

while an insect between 4 and 5 days old would receive a value of 5, and so on. Because of the different development times required for males and females, especially in the sixth instar (McGugan, 1954), the maximum value a male insect could receive is 7 and the maximum value a female could have is 10 (Mulye and Gordon, 1990).

A paired t-test ($p < 0.05$) was used for each solution to determine whether the palp used (left vs. right) had an effect on the response. No significant difference was found between the palps (Table 5), and only one palp was randomly selected for the rest of the analyses. A paired t-test ($p < 0.05$) was also used to determine if there was a significant difference between the initial response to sucrose at the beginning of each trial compared to the sucrose response at the end of each trial. There was no significant difference between the two (paired t-test; $t = -1.96$, $n = 52$, $p = 0.055$), therefore the sensillum's response did not drastically deteriorate during the course of experimentation. However the p value was very close to 0.05 indicating that the length of time that the insect or solutions are kept in use during experimentation could have the possibility of influencing the results. A repeated measures ANOVA ($p < 0.05$) was utilized for each solution to see if age or sex had an effect on the insect response, or if the two interacted with each other. Both age and sex were found not to have an effect, nor did they show an interaction for any of the solutions tested. A two-sample t-test ($p < 0.05$) was performed between experiment 6 ($n = 52$), where the insects were starved prior to experimentation, and experiment 7 ($n = 15$), a similar experiment where insects were not starved prior to experimentation, to see if starvation had an affect on the mean impulses/s of sucrose. The results of the two-sample t-test indicated that starvation had no effect on the mean

impulses/s of sucrose ($t = 0.974$, $n = 67$, $p = 0.334$). Thus the nutritional status of the insect also did not influence its response.

Table 5: Effect of various variables on response of L1 sensillum, $\alpha = 0.05$, NA = not applicable.

Variable Tested	Statistical Test Used	n	p	F	t
Difference between palps	Paired t-test	15	Sucrose (1st hit) = 0.107	*NA*	1.723
			D-xylose = 0.767	*NA*	-0.302
			Raffinose = 0.182	*NA*	1.405
			Sucrose (last hit) = 0.924	*NA*	0.097
Difference between sucrose stimuli	Paired t-test	52	0.055	*NA*	-1.96
Effect of age	Repeated Measures ANOVA	52	Sucrose (1st hit) = 0.844	0.48	*NA*
			D-xylose = 0.792	0.55	*NA*
			Raffinose = 0.351	1.16	*NA*
			Sucrose (last hit) = 0.776	0.57	*NA*
Effect of sex	Repeated Measures ANOVA	52	Sucrose (1st hit) = 1.00	0.00	*NA*
			D-xylose = 1.00	0.00	*NA*
			Raffinose = 1.00	0.00	*NA*
			Sucrose (last hit) = 1.00	0.00	*NA*
Effect of starvation	2-sample t-test	Experiment 6 n=52 Experiment 7 n=15	0.334	*NA*	0.974

Since all 52 insects responded to sucrose, but only some responded to D-xylose and/or raffinose, the insects were grouped into four categories based on which solutions elicited a response. The percentages of the insects in each category are listed in Table 6.

Table 6: Number of insects and percentages grouped into categories by L1 sugar-sensitive cell response to solutions; n = 52.

Category	Number of Insects Responding (out of 52)	Percent Responding
1 *sucrose only*	20	38.5
2 *sucrose and D-xylose*	8	15.4
3 *sucrose and raffinose*	6	11.5
4 *sucrose, D-xylose, and raffinose*	18	34.6

Most insects that responded to D-xylose also responded to raffinose (34.6%), therefore a greater proportion of insects responded to both pyranose sugars (Category 4), then to D-xylose (Category 2) or raffinose (Category 3). There was also a slightly higher percentage of insects in Category 2 (15.4%) than in Category 3 (11.5%).

Yet, when looking at the order in which the solutions were tested, a response to raffinose was never seen when it was presented in Order 1 (i.e. sucrose, D-xylose, raffinose, sucrose). In addition, the mean response to D-xylose was higher when it was tested directly after sucrose (Order 1), as opposed to sucrose being tested followed by raffinose and then by D-xylose (Order 2). The same pattern was seen for raffinose, the mean number of impulses/s was higher when it was tested right after sucrose, in Order 2 (Table 7). A Mann-Whitney U test (p < 0.05) was used because the data for xylose was not normal, it revealed that the difference between order was significant for xylose (z = -2.00, n = 26, p = 0.045). An unpaired t-test (which was used on the data from raffinose because this data was normal) gave the opposite for raffinose (t = -0.296, n = 24 , p = 0.77). Thus, the order in which xylose is used seems to have an effect on the response.

Table 7: Mean impulses/s (±S.E.) of L1 sugar-sensitive cell response to D-xylose and raffinose for categories 2-4 only. Category 2 = responds to sucrose and D-xylose, Category 3 = responds to sucrose and raffinose, Category 4 = responds to sucrose, D-xylose and raffinose; Order1 = sucrose, D-xylose, raffinose, sucrose, Order 2 = sucrose, raffinose, D-xylose, sucrose.

# of Insects	Stimulus	Category	Order	Mean Impulse/s (±S.E.)
2	D-xylose	2	1	21.17 (6.65)
6	D-xylose	2	2	2.5 (1.5)
6	Raffinose	3	2	14.5 (4.70)
0	Raffinose	3	1	0
11	D-xylose	4	1	27.4 (6.04)
7	D-xylose	4	2	12.62 (3.39)
11	Raffinose	4	1	14.5 (2.97)
7	Raffinose	4	2	15.75 (3.53)

Experiment 7: L1 Sensillum Response to D-xylose and Raffinose Before and After Testing with Sucrose

If order had no effect on the response of the L1 sugar-sensitive cell, then responses to the pyranose sugars should be seen both before and after testing with sucrose. To see if order had an effect, 25 mmol/L concentrations of sucrose, xylose, and raffinose were used. 25 mmol/L KCl served as the solvent and also as the control solution. At first xylose, raffinose, and KCl were randomly tried on the insect before any testing with sucrose was done. Only once xylose, raffinose, and KCl had each been tested was sucrose tested, followed again by xylose, raffinose, and KCl. Each subsequent testing (in random order) of xylose, raffinose, and KCl was always three minutes after testing with sucrose.

The results obtained in Table 8 show that at no time did D-xylose or raffinose elicit a response when there was no prior testing with sucrose. A response was seen, however, after testing with sucrose. The mean impulses/s increased from 0 to 2.9 for D-xylose and from 0 to 8.3 for raffinose after testing with sucrose. Even KCl showed an increased response, from 0 impulses/s prior to testing with sucrose to 1.9 impulses/s following testing with sucrose. However, not all insects responded after testing with sucrose. While there was a significant difference between the response to raffinose before and after testing with sucrose according to the paired t-test ($t = -2.83$, $n = 18$, $p = 0.012$), there was no significant difference in the responses of xylose (paired t-test, $t = -1.42$, $n = 18$, $p = 0.17$) and KCl (paired t-test, $t = -1.0$, $n = 11$, $p = 0.42$).

Table 8: Mean impulses/s ($\pm$S.E.) of L1 sugar-sensitive cell response to solutions tested before and after sucrose, (n = 18) for D-xylose and raffinose, (n = 11) for KCl. Mean impulses/s for sucrose = 53.9 ($\pm$5.9); n = 15 (not shown).

Solutions Tested (25 mmol/L)	Mean impulses/s before Sucrose Tested	Mean impulses/s after Sucrose Tested ($\pm$S.E.)	Number of insects responding after testing with Sucrose
D-xylose (25 mmol/L KCl solvent)	0	2.9 (2.0)	2 of 18
Raffinose (25 mmol/L KCl solvent)	0	8.3 (3.0)	7 of 18
KCl	0	1.9	1 of 11

However, since there was absolutely no response to any of the pyranose sugars applied prior to testing with sucrose, and an increase in the mean impulses/s after testing

with sucrose, it seemed to indicate that the order in which the solutions are presented may have had some effect, especially with regards to raffinose.

Experiment 8: L1 Sensillum Response to D-xylose and Raffinose after Testing with 25 mmol/L KCl Containing 25% Methanol

Occasionally, the pore at the tip of a taste sensillum extrudes a viscous substance (Stürckow, 1971) containing acid mucopolysaccharides that are secreted by the trichogen and tormogen cells (Pietra et al., 1980). This substance separates the external environment at the tip of the sensillum from the internal environment of the dendrites (Pietra et al., 1978). According to Pietra et al. (1978), the features of the apical mucopolysaccharides can influence the flow of substances reaching the dendrites, acting as a barrier and modulating the effect of the stimuli. It is possible that the mucopolysaccharides reduce the effect of the pyranose sugars, which do not seem to stimulate the L1 sugar cell as strongly as sucrose, to sub-threshold stimulation, resulting in a lack of response. To see if this was the case, 25 mmol/L solutions of xylose and raffinose in 25 mmol/L KCl were tested, with 25% methanol in 25 mmol/L KCl as the control solution. The control solution was applied to the sensillum prior to testing with the pyranose sugars. The methanol present in the solution should help dissolve the mucopolysaccharides at the pore tip (Whistler and Smart, 1953; Brimacombe and Webber, 1964), thus allowing the pyranose sugars unobstructed access to the dendritic receptors. The methanol solution was applied for approximately 3 minutes, after which the xylose and raffinose solutions were randomly tested. 25 mmol/L sucrose in 25

mmol/L KCl was applied at the end to make sure the cell had not died due to the influence of methanol.

No response was seen from any of the pyranose sugars following tests with the 25% methanol KCl solution, but a response was still obtained from the sucrose control indicating that the cell was still functional after testing with the methanol solution.

Experiment 9: L1 Sensillum Response to D-xylose and Raffinose in a Solvent Containing 25% Methanol

Since there is a three minute disadaptation period between stimulations, it is conceivable that more mucopolysaccharides secreted by the accessory cells could have re-blocked the pore during the time following stimulation with the KCl methanol solution. To be certain that a blockage in the apical pore of the sensillum was not responsible for the lack of a response, 25% methanol was used as a solvent for all the solutions; 25 mmol/L KCl in 25% methanol served as the control solution. The test solutions were 25 mmol/L concentrations of xylose, raffinose, and sucrose. If the cell can respond to 25 mmol/L sucrose dissolved in 25% methanol, and possessed the ability to respond to pyranose sugars, then a response should also be obtained from dissolving 25 mmol/L of D-xylose and raffinose in the same solvent. To determine if the sucrose solution in 25% methanol had an effect on the cell, a control solution of 25 mmol/L sucrose (dissolved in 25mmol/L KCl, no methanol) was used as a comparison.

A paired t-test ($p<0.05$) was used to determine if there was a difference between the responses to sucrose dissolved in 25 mmol/L KCL with 25% methanol, versus sucrose dissolved only in 25 mmol/L KCl. The results obtained from the two solutions were not significantly different ($t = -0.940$, $n = 5$, $p = 0.400$), but no response was obtained from the D-xylose or raffinose methanol solutions (Table 9). The lack of response after testing with the methanol solutions indicates that a mucopolysaccharide pore plug was not responsible for the lack of response to the pyranose sugars, since the methanol in the solution should have dissolved these polysaccharides during stimulation (Whistler and Smart, 1953; Brimacombe and Webber, 1964). The results also indicate that the L1 sugar-sensitive cell does not have the ability to respond to any of the pyranose sugars tested, unlike the LST, nor does it possess a P-site such as that described by Shimada (1987). The sugar-like responses elicited by the pyranose sugars in the previous experiments were most likely due to residual sucrose left on the sensillum after the initial testing with sucrose.

Table 9: Mean impulses/s (±S.E.) of L1 sugar-sensitive cell responses to solutions with/without 25% methanol in 25 mmol/L KCl solvent.

Response to 25 mmol/L Solutions (25 mmol/L KCl Solvent)				
Sucrose (±S.E.)		D-xylose	Raffinose	KCl
Without 25% Methanol	With 25% Methanol	With 25% Methanol	With 25% Methanol	With 25% Methanol
59.4 (±23.1) impulses/s ($n = 5$)	73 (±30.7) impulses/s ($n = 9$)	None ($n = 5$)	None ($n = 5$)	None ($n = 5$)

Experiment 10: Comparison Between L1 and LST Sensillum Responses to Fructose

Since the L1 sensillum shows differences in response compared to the LST sensillum to such important sugars as sucrose (Albert, 2003), glucose, and other pyranose sugars, it follows that the L1 response to furanose sugars might differ from the LST as well. A response to fructose, another important sugar to the spruce budworm (Heron, 1965; Harvey, 1974; Albert et al., 1982; Albert and Jerrett, 1981; Panzuto et al., 1997), has already been shown (see experiment 1). However a dose-response curve to fructose for the LST sensillum, and a comparison between the LST and the L1 sensilla regarding this sugar, has not been done to date. Solutions of 5, 50, and 500 mmol/L D-fructose dissolved in 25 mmol/L KCl were applied to both the L1 and LST of the same maxilla on each insect. Only insects with both a functioning L1 and LST sensillum were used.

The responses between the L1 and LST sensilla to each solution tested were compared using a two-way ANOVA. An ANOVA was also used to determine if stimulus concentration had an effect on response of the LST sensillum. A Bonferroni multiple comparison test was utilized to determine differences among stimuli in order to ascertain the concentration at which the LST sugar-sensitive cell response plateau to fructose is reached. To observe if increasing concentration resulted in increasing firing frequency, the average of the responses (mean impulses/s) and the standard error (S.E.) were plotted against the concentration of the solutions used for both the L1 and LST.

Table 10 gives the mean impulses/s (± S.E.) of the L1 and the LST response to fructose. It appears that the L1 sensillum is much more sensitive to fructose than the LST

because not only does L1 respond to a lower D-fructose concentration than the LST (5 mmol/L), but it also responds with higher mean impulses/s to the other concentrations tested. The ANOVA performed on the stimulus concentration and response of the LST indicates that stimulus concentration has an effect on the response (df = 2,89; F = 54.61; p < 0.0001). The Bonferroni multiple comparisons test demonstrated that the responses to 5, 50, and 500 mmol/L concentrations all differed from each other, indicating that the LST response does not plateau at the 500 mmol/L concentration. A higher concentration would have to be tested in order to ascertain at what concentration the LST response to D-fructose reaches a plateau.

Table 10: Mean response (impulses/s) of L1 and LST sensilla (±S.E.) to three fructose concentrations; n = 15.

Fructose Concentration	Mean impulses/s (± S.E.)	
(mmol/L)	L1	LST
5	13.2 (3.87)	0
50	49.5 (4.26)	16.4 (2.14)
500	77.8 (4.53)	41.9 (3.83)

Figure 5 shows the mean impulses/s vs. concentration for both the L1 and the LST. It is clear from this graph that the response of the LST to fructose is much lower than the response of the L1, and a two-way ANOVA verifies that the response between the L1 and the LST sensilla significantly differ from each other for all concentrations tested (df = 1,89; F = 90.99; p < 0.0001).
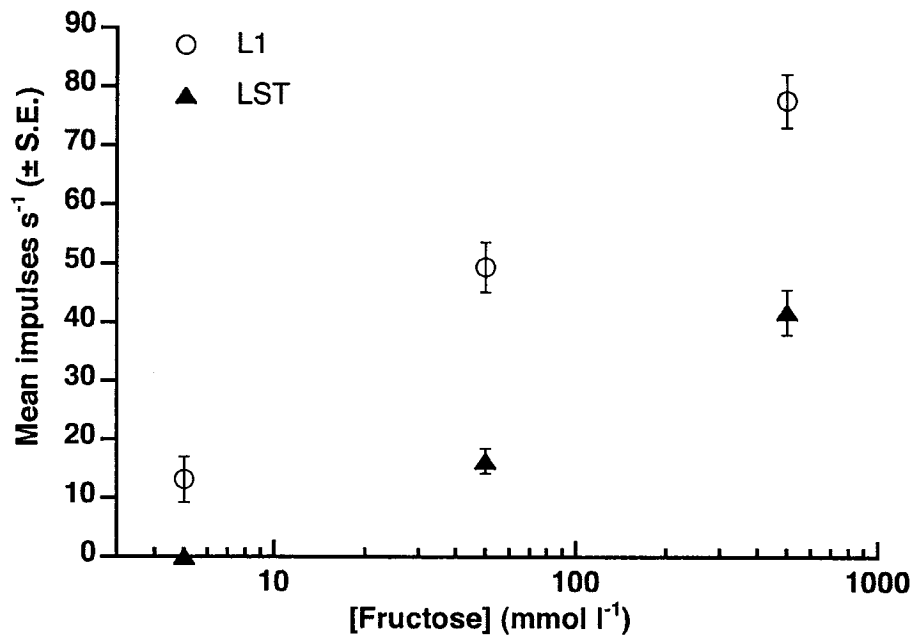
Fig. 5: L1 and LST sensilla responses to three fructose concentrations, n=15.

Unfortunately, a double-reciprocal (Lineweaver-Burk) plot could not be obtained for the LST response to fructose, because only 3 concentrations were tested, one of which gave a response equal to 0 (see Table 10). Thus the K_b and V_{max} values for the LST response to fructose were unattainable.

Table 11 gives a comparison of the threshold, plateau, K_b, and V_{max} (for 100 and 500 ms) values obtained from the L1 sensillum in response to fructose (from experiment 1) with those obtained previously by Albert and Parisella (1988b) and Albert (2003) for the L1 and LST sugar cell responses to sucrose. The adjusted values corresponding to the amount of fructose that is actually stimulating (i.e. 20%) are also given, as are the

threshold and plateau values for the LST response to fructose obtained from experiment 10. The L1 has a lower threshold then the LST to fructose. However, the LST sucrose response has a lower threshold, plateau, and K_b than the L1 sensillum. Thus, according to the results, the L1 sensillum is more sensitive to fructose than the LST, but the LST is more sensitive to sucrose than the L1 sensillum.

Table 11: Response characteristics of L1 and LST of sixth instar spruce budworm larvae to the given concentrations of sucrose and fructose and the adjusted concentrations (20%) of fructose (data for LST and L1 response to sucrose from Albert and Parisella, 1988b; and Albert, 2003).

Sensillum	V_{max} at 100ms (impulses/s)	V_{max} at 500ms (impulses/s)	K_b (mmol L^{-1})	Threshold (mmol L^{-1})	Plateau (mmol L^{-1})
L1 (fructose)	175.4	100	7.6-9.5	5 – 50	>500
LST (fructose)	unknown	unknown	unknown	5 < X <50	>500
L1 (sucrose)	200	105	20 – 25	1 – 10	200
LST (sucrose)	201	110	1.5 – 1.8	< 0.5	50

Discussion

In the sixth instar spruce budworm, the L1 sensillum on the maxillary palp possesses a sugar-sensitive cell that responds to sucrose and to furanose sugars such as fructose. Pyranose sugars also seemed to elicit a response from the L1 sugar-sensitive cell, but on closer investigation this was not found to be the case. None of the pyranose sugars I tested elicited any response from the sugar-sensitive cell, and the effect previously attributed to them is believed to be due to residual sucrose remaining on the sensillum during experimentation. Except in experiments 7 and 8, each insect was initially tested with sucrose to ensure that the insect preparation was alive and functioning. It may be that some sucrose was left on the L1 sensillum after testing because of its small size (3-5 μm long, 2 μm in diameter) (Albert, 1980), and upon testing with subsequent solutions the L1 sensillum was thus not stimulated by the pyranose sugars but responded to the residual sucrose that was still present on the sense organ. A control for this problem could be used to verify the results; one possible control would be to wash the sensillum between stimulations. This can be done by filling a micropipette with distilled water and applying it to the sensillum in the same way as one would do when testing a solution. A control such as this would, however, be more time consuming and require extra manipulations. Since the residual sucrose left on the sensillum only gives an average increase in response of 8.3 impulse/s, it does not exert a great influence on the results. The presence of residual sucrose left on the L1 sensillum is a possible effect that can shape results, perhaps more so when dealing with low concentrations of the stimulus, but the added increase in impulses/s is low enough that it

may not be necessary to perform extra manipulations, especially since the increase seems to occur only following stimulations with sucrose. Based on these results, it is concluded that the L1 sensillum of the spruce budworm does not have pyranose sites (such as that described in flies) and does not show variability in response to these sugars, but rather shows no response to pyranose sugars at all.

In flies, the sugar cell is known to possess both P-sites for interacting with pyranose sugars as well as F-sites for binding with furanose sugars (Shimada, 1974). All pyranose sugars used in this study are known to stimulate the P-sites in flies (Shimada, 1987), as well as the sugar-sensitive cell in the LST of both fourth and sixth instar spruce budworms (Panzuto and Albert, 1997), except for melezitose which was not tested in Panzuto and Albert's study. The fact that the L1 sugar-sensitive cell responds to fructose and not to any of the pyranose sugars tested suggests that this cell only contains F-sites. There have been other instances where only one type of sugar elicited a response from the sugar cell (Hansen, 1978). For example, in the caterpillar *P. rapae* the sugar-sensitive cell in the MST responds to sucrose and fructose, among other sugars, but the sugar-sensitive cell present in the LST does not respond to fructose, and the sugars it does respond to are all pyranoses (Schoonhoven and Van Loon, 2002). In *Pieris brassicae*, the sugar-sensitive cell present in the LST responds to both furanose and pyranose sugars, while the sugar cells in the MST and epipharyngeal sensilla respond only to glucose and sucrose (Ma, 1972). In the spruce budworm, xylose, myo-inositol, raffinose, 1-0-methyl-D-α-glucopyranoside, as well as several furanose sugars elicit a response from the LST sugar-sensitive cell (Panzuto and Albert, 1997), indicating the presence of both P- and F-

sites in this cell. Furthermore, a feeding response from sixth instar larvae was seen for all the pyranose sugars that elicited a response from the LST sugar-sensitive cell, except for L-arabinose which was found not to stimulate feeding (Albert, 1982).

Raffinose is a readily utilizable sugar to the spruce budworm (Harvey, 1974); it stimulates feeding (Albert et al., 1982) and it is one of the most acceptable sugars to this insect (Heron, 1965). Raffinose is one of the most widely occurring soluble sugars in plants, possibly second only to sucrose (Dey, 1990); it is also one of the main sugars present in both spruce and balsam fir during the winter. However, raffinose is not present in appreciable amounts during the summer (Neish, 1958; Little, 1970). Younger instars may encounter some raffinose in early spring, but sixth instar larvae are unlikely to find this sugar during the time when they are actively feeding, since it only begins to slowly increase in August (Little, 1970). So although raffinose is a stimulating and acceptable sugar to the budworm, its role in the feeding behaviour of sixth instar larvae under normal conditions in nature may be negligible, due its low levels in host foliage during the summer, which might explain the lack of sensitivity of the L1 sugar cell to this carbohydrate. However, due to the fact that this sugar is widely distributed and may increase in times of stress, having at least one cell that responds to this sugar (i.e. the LST sugar-sensitive cell) when it is present would be advantageous to the insect.

Other sugars not normally encountered by sixth instar budworm larvae during feeding are arabinose and xylose (Heron, 1965), which are found in small quantities in coniferous foliage during the Fall and Winter months (Assarsson and Theander, 1958).

These sugars are also not the most favoured pyranose sugars to sixth instar spruce budworms, since in behavioural tests myo-inositol, raffinose, and 1-0-methyl-α-D-glucopyranoside were all preferred over xylose, which was the least preferred of the stimulatory sugars, and L-arabinose, which was found to be non-stimulatory (Albert et al., 1982). In sixth instar larvae, myo-inositol, raffinose, and 1-0-methyl-α-D-glucopyranoside gave the highest electrophysiological responses of the pyranose sugars, while the firing frequencies of L-arabinose and xylose were among the lowest (Panzuto and Albert, 1997). Melezitose, L-arabinose, and xylose were found by Harvey (1974) to have low utilizability, and the D-isomer of arabinose is more behaviourally stimulating to the budworm then the L-isomer (Heron, 1965). Heron observed only a slight feeding response to melezitose, while L-arabinose and xylose did not elicit any substantial feeding. Moreover, Albert and Jerrett (1981) found that budworms reared on diets containing arabinose produced the least amount of dry weight of frass, and, as mentioned earlier, L-arabinose was found to be non-stimulatory in feeding preference tests conducted by Albert et al. (1982) while xylose was the least preferred of the stimulatory sugars. Both arabinose and xylose are pentose sugars, which are generally assumed to have no nutritional value to most insects (Dadd, 1985). It would be a waste of resources to devote additional sensory equipment to carbohydrates that have little or no nutritional value to the insect.

The sugar alcohol myo-inositol also did not elicit a response from the L1 sugar-sensitive cell. This is opposite of what is found in the LST where it is the third most preferred sugar of sixth instar larvae, both behaviourally and electrophysiologically

(Albert et al., 1982; Panzuto and Albert, 1997). Myo-inositol, present in small amounts in conifer needles during Fall and Winter (Assarsson and Theander, 1958), is a ubiquitous plant constituent and plays an important role in plant metabolism and osmoregulation (Loewus and Murthy, 2000; Nelson and Bernays, 1998). According to Loewus and Murthy (2000) this sugar alcohol is involved in plant structure and function as well as in plant stress-related responses; additionally it can be synthesized from glucose and take part in the biosynthesis of raffinose. It may also be present in plant leaves at the same levels as the principal leaf sugars, i.e. sucrose, fructose, and glucose (Nelson and Bernays, 1998). Myo-inositol could also be related to the level of nutrients present in plants, such as the levels of protein or carbohydrates, or it may just be a general indicator of suitable plant material (Nelson and Bernays, 1998). Inositol is found in insect cell membranes, and may act as a second messenger. Some insects, such as *B. mori*, require this sugar alcohol, while others, like *M. sexta*, use it as carbohydrate source (Nelson, 1996). Though non-stimulatory to the L1 sensillum, myo-inositol may be important to the spruce budworm as evidenced by the preference of the insect to this sugar as well as by the high response of the sugar-sensitive cell in the LST to it.

Since the LST already shows a response to glucose, xylose, myo-inositol, 1-0-methyl-D-α-glucopyranoside, raffinose, and L-arabinose, at least at the 25mmol/L concentration (Panzuto and Albert, 1997), it may seem redundant to have a second sugar cell that would also respond to these same sugars. While there have been examples of other Lepidopterans that possess more than one sugar-sensitive cell that respond to some of the same sugars (i.e. *P. brassicae*, *B. mori*), the response characteristics of these cells

to the sugars differ (Schoonhoven and Van Loon, 2002). This is also the case with the LST and L1 responses to sucrose (Albert, 2003) and fructose in the spruce budworm. The lack of a response from the L1 sugar-sensitive cell to pyranose sugars could reflect the level of importance that these sugars have for sixth instar larvae. Most of the pyranose sugars tested, except for glucose, are not present in large amounts in mid-June, when sixth instar larvae are actively feeding. Thus, there may not be a need to have two cells responding to sugars that, under normal conditions, are not present in summer at the time when the insect is feeding, even if some of these sugars are stimulatory and can be utilized. However, the utilizability of most of these sugars to the spruce budworm (Harvey, 1974) suggests that the ability to sense these sugars when they are present in the plant, such as during times of stress, would be an advantage to the insect. Moreover, all phytophagous insects possess sugar-sensitive cells, and all plants produce large amounts of sugars from photosynthesis (Chapman, 2003), therefore sugars can indicate a suitable energy source (Nelson and Bernays, 1998). Given that some of these pyranose sugars are primary plant metabolites and widely distributed, having at least one cell that responds to them would be an asset to the insect, especially since sugars are important phagostimulatory components (Chapman, 2003). Detecting sugar as a token stimulus is the hypothesized role of the LST sugar-sensitive cell (Albert, 2003), and this might explain the broad range of carbohydrates that this sensillum can respond to.

D-glucose, D-fructose, and sucrose are primary metabolites, and are all free sugars found in the summer foliage and staminate flowers of white spruce (Heron, 1965). These carbohydrates are present in increased amounts during the winter and are widely

distributed among plants; moreover they act as phagostimulants for many herbivorous insects (Schoonhoven and Van Loon, 2002). Sucrose and fructose are the most stimulating sugars to spruce budworms according to Heron (1965), while D-glucose was found not to induce feeding. However, Harvey (1974) reported good growth, and therefore utilizability, of diets containing glucose by spruce budworms, and attributed the poor feeding response to the low palatability of this sugar. Preference for ß–D-glucose in sixth instar larvae was low (Albert et al., 1982) and insects reared on diets containing sucrose and fructose also produced more dry weight of frass then those reared on glucose; however these differences were not significant (Albert and Jerrett, 1981). The low stimulatory effect of D-glucose on the LST when compared to fructose and sucrose, and the lack of a response to D-glucose from the L1 is in agreement with the above behavioural findings, that glucose, although utilizable, is not a very stimulatory sugar to spruce budworms.

The electrophysiological response of the LST of sixth instar spruce budworms was found to be closely correlated with the behavioural preferences for sucrose and fructose (Albert et al., 1982). The L1 sensillum of the maxillary palp of sixth instar larvae is also known to respond to sucrose and fructose, however the L1 response to sucrose differs from that of the LST (Albert, 2003), as does the response to fructose.

The sucrose level in the budworm's most preferred host, balsam fir, in current year fir foliage during the period when sixth instar larvae are feeding is about 37 mmol/L (Little, 1970), and the preferred behavioural concentration range of sucrose (10-50

mmol/L) of sixth instar larvae correlated closely with this (Albert et al., 1982). The amount of fructose present in current year foliage of balsam fir during the same time is approximately 3 milligrams per gram of oven-dried weight (Little, 1970). The moisture content in current year foliage is about 75-80% (Albert and Parisella, 1988b). This gives a foliar concentration of 4.2 mmol/L fructose in current year foliage. The moisture content of mature balsam fir foliage is 51%, and the fructose level in 1-year-old foliage during mid-June is approximately 5 milligrams per gram of oven-dried weight (Little, 1970). These values give a fructose concentration of 26.7 mmol/L in older foliage. The threshold of the L1 sensillum to fructose is between 5 and 50 mmol/L, similar to that of the LST in *B. mori* which has a threshold of 5-10 mmol/L (Schoonhoven and Van Loon, 2002). The 4.2 mmol/L concentration of fructose found in current-year foliage is very close to 5 mmol/L, so the L1 sugar-sensitive cell can most likely detect the levels of fructose present in current-year foliage. However, the levels of fructose in older foliage, which sixth instar larvae prefer (Bauce et al., 1994), are well within the sensitivity range of the L1 sensillum. According to electrophysiological tests performed by Panzuto and Albert (1997), the LST responds to 25 mmol/L fructose concentrations both behaviourally and electrophysiologically (Albert et al., 1982; Panzuto and Albert, 1997). This value is below that of the concentration of fructose found in mature needles and therefore fructose levels in older foliage are well within the sensitivity range of the LST as well. This implies that while the LST cannot sense the fructose levels present in current-year foliage, both the L1 and the LST can sense the levels of fructose present in older foliage.

Since only 20% of fructose is in the fructofuranose form and able to stimulate the F-sites in a sugar cell (Cheung and Smith, 1998), only 20% of the fructose concentrations used elicited the responses seen. Therefore the K_b is actually 9.5 mmol/L for the first 100 ms of the response to fructose, and 7.6 mmol/L for the first 500 ms of the response; however the V_{max} stays the same (Table 11). Unfortunately, no K_b value or V_{max} was obtained for the LST response regarding fructose, thus a comparison between the two sensilla regarding these values can not be made here. The K_b for the L1 response to fructose (7.6-9.5 mmol/L) is lower then its response to sucrose (20-25 mmol/L), but still not as low as the LST response to sucrose (1.5-1.8 mmol/L). Thus it appears that the L1 sensillum has a greater affinity for fructose than sucrose, but the LST's affinity for sucrose is still greater than the L1 sensillum's affinity for fructose, or sucrose for that matter (i.e. K_b LST sucrose < K_b L1 fructose < K_b L1 sucrose). The V_{max} for fructose at the 500 ms period does not differ much from that of the L1 and LST values obtained for sucrose, but the V_{max} of both the LST and the L1 sensilla to sucrose is greater then that of the L1 sensillum to fructose for the first 100 ms.

According to Albert and Parisella (1988b), solutions of sucrose that elicit a greater firing rate from the sugar-sensitive cell in the LST are preferred, and this correlates well with the insect's feeding behaviour. If preferred sugars elicit a higher firing frequency, then based on the results of this study sucrose should be preferred over fructose, at least when comparing the first 100 ms of response of the LST to that of the L1 sensillum. This is in agreement with the behavioural results that sucrose is the most preferred sugar for sixth instar larvae, followed closely by fructose (Albert and Jerrett,

1981; Albert et al., 1982; Panzuto and Albert, 1997). This also correlates well with the low concentration of fructose found in both current-year and mature balsam fir foliage compared to the concentration of sucrose (Little, 1970). Additionally, sucrose is usually always a more effective stimulus then fructose in many lepidopterous species (Schoonhoven and Van Loon, 2002).

Raffinose, fructose, sucrose, glucose, and total sugar levels rise in plants as temperatures cool (Little, 1970). Their use as osmoregulators may not be limited to times of cold stress, but they may also function as osmoregulators in other stressful conditions, such as during drought. In water-stressed plants, such as black spruce, ß-glucose is known to increase to more then 5 times the normal amount (Zwiazek and Blake, 1990). If the role of L1 is to detect water stress in plants, as was hypothesized by Albert (2003), then it would be an advantage for the insect to be able to recognize glucose, a sugar that increases so drastically during times of stress. However, the response characteristics of the LST sugar-sensitive cell to glucose have not yet been ascertained, and due to the high levels of glucose in new foliage (39.5 mmol/L) as well as the importance of the LST sensillum in feeding (Albert and Parisella 1988b); the LST may already possess the ability to sense large amounts of glucose. Moreover, ß-D-glucose is not the most stimulatory sugar, both electrophysiologically and behaviourally (Panzuto and Albert, 1997; Albert et al., 1982). Thus glucose, though a good carbohydrate source for the spruce budworm, may not be as important as fructose or sucrose, the two most preferred sugars of sixth instar larvae. Furthermore, because of glucose's low palatability, it may not warrant a second cell devoted to its detection. In nature, glucose usually always

occurs in the presence of sucrose and/or fructose, it is not found in isolation from the other two primary plant metabolites, and its lack in inducing feeding may be irrelevant because of this (Harvey, 1974). Thus its failure to elicit a stimulatory response from the L1 sensillum may be insignificant, since this sensillum is capable of detecting the two other sugars that are found in conjunction with glucose. Alternatively, it may not be necessary for the insect to sense all sugars that increase in stressed plants. If the L1 sugar-sensitive cell can sense a few of the sugars that show large increases in stressed plants, such as fructose + α-galactose which show more than a 7 fold increase (Zwiazek and Blake, 1990), then that may be sufficient to inform the insect that the plant is under stress.

The lower threshold of the L1 sensillum compared to the LST implies that the L1 is more sensitive to fructose then the LST, which is opposite of what was found for sucrose (Albert, 2003). The L1 plateau to fructose seems to be above the 500 mmol/L concentration, and the LST plateau is also above the 500 mmol/L concentration. Thus it would seem that L1 and the LST are both perfectly suited to detect the levels of fructose normally found in host-plants as well as those found in stressed plants. Furthermore, while it has been shown that the L1 sensillum has a higher threshold then the LST to sucrose, the levels of this sugar in black spruce actually decrease slightly when the plant is under water stress. Nonetheless, it should be noted that not all trees may experience the exact same increases or decreases in carbohydrates as black spruce.

The ability to detect water stress in plants may be advantageous to the insect, since detecting higher than normal sugar levels in plants may increase food intake or the biting rate of insects. According to Shiff et al. (1989) there is generally a positive correlation between biting rate and the relative utilizability of carbohydrates, as shown in the caterpillar *Heliothis zea*. Moreover, a higher quality food that increases growth rate and reduces the time required to reach pupation would thereby decrease exposure to predators and increase survival and fecundity (Thomas, 1989).

In conclusion, there is a difference in structure between the L1 and LST sugar-sensitive cells, since their responses, or lack thereof, to certain sugars indicate that they possess different receptor sites. The LST responds to both pyranose and furanose sugars, therefore possessing P- and F-sites, while the L1 sensillum only responds to furanose sugars, indicating that only F-sites are present. However, care should be taken when comparing the P- and F-sites found in flies to other insects; the sugar-receptors in spruce budworms may not behave or be structured at all like those found in flies. Both the responses to sucrose and fructose in the LST correlate closely with behavioural observations of Albert et al. (1982), as does the concentration of sucrose found in current year host foliage (Little, 1970). The fructose concentration in mature foliage corresponds well with the range of sensitivity of both the LST and the L1 sensilla to this sugar. This is in agreement with the behavioural data that sucrose is a more powerful phagostimulant as well as the most preferred sugar, followed closely by fructose, especially when considering the input of the LST as the primary one influencing feeding behaviour. Though the function of the L1 sensillum in relation to the LST is not clear, it is apparent

that the response characteristics of both sensilla correspond closely to the physiology of the spruce budworm's host plant, and both sensilla are likely to have an important role in food discrimination.

Further investigation on the influence of the L1 sensillum on feeding behaviour is needed in order to gain greater knowledge of its function and that of the maxillary palp. Ablation experiments have been useful in providing insight as to the role of gustatory sense organs in various lepidopterans. For example, Blom (1978) found that ablation of the maxillary palps in *Pieris brassicae* resulted in an increase in intake of sucrose at low concentrations, but had no effect at high concentrations. He hypothesized that the maxillary palps affect the uptake of food in a general, possibly in an inhibitory way. In *Pieris brassicae*, all three sugar-sensitive cells present in the LST, MST, and epipharyngeal sensilla are required for a normal behavioural response to sucrose (Blom, 1978). It is likely that a similar situation exists within the spruce budworm, where both the L1 and LST sugar-sensitive cells are needed for proper food discrimination. Ablation experiments of the LST and the maxillary palp would be helpful in defining the role of both sensilla, but due to the insect's small size performing these experiments would be extremely difficult.

More information is also needed on the response characteristics of the LST to various sugars, such as fructose. It would be interesting to compare the K_b and V_{max} of the LST response to fructose to that of the L1, as well as to the sucrose responses of both sensilla. Behavioural tests should also be performed; the behavioural threshold to fructose

as well as its preferred concentration range would be of interest in determining the correlation between the behavioural response and the electrophysiological data.

Knowledge of how a host-plant's chemicals are perceived by an insect is important in unraveling feeding behaviour. Thus, in order to implement effective control methods against insect pests, such as the spruce budworm, an understanding of its feeding behaviour and the underlying mechanisms that influence this is essential (Dethier, 1969). The results of this thesis cannot be applied directly as pest management strategies; however they help in discerning the basis of feeding behaviour, an understanding of which is crucial in developing successful forest-pest management strategies.

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