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STRUCTURE, DISTRIBUTION AND INNERVATION OF SENSILLA ON THE

OVIPOSITOR OF THE SPRUCE BUDWORM *CHORISTONEURA*

*FUMIFERANA,* AND ELECTROPHYSIOLOGICAL RESPONSES FROM TYPE

II SENSILLA

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# ABSTRACT

**Structure, distribution and innervation of sensilla on the ovipositor of the spruce budworm *Choristoneura fumiferana*, and electrophysiological responses from Type II sensilla**

Neil Banga

Scanning and transmission electron microscopy techniques were used to perform this first investigation of the ovipositor of *Choristoneura fumiferana*. The female spruce budworm has four types of trichoid sensilla on its ovipositor. A unique and rare type of sensillum is described. This Type II sensillum is multiporous and also has a single pore at the tip. The other sensillar types found were: Type I and III sensilla, which are short and long multiporous hairs respectively, and Type IV sensilla which are long, aporous hairs. Type I and III hairs are probably olfactory, while Type IV hairs are probably mechanosensory. Type II hairs are innervated by 4 dendrites. Physiological responses were obtained from only these Type II sensilla using the tip recording method. Behavioural studies have shown that mated insects are encouraged to oviposit in the presence of host waxes whereas virgins are not. The response of Type II hairs to waxes in solution from their most preferred host, white spruce (*Picea glauca*), revealed no differences between unmated and mated insects. Mated and unmated females' sensilla responded differently to common polar nutrients. Results from this study suggest that mated females may be using polar compounds rather than the surface waxes to locate potential hosts.

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# INTRODUCTION

Oviposition behaviour is extremely important in lepidopterans because most juveniles are relatively immobile and rely upon the adult of the species to select a proper host plant. The process of host selection is divided into several steps: search and orientation, landing and surface evaluation, and finally acceptance or rejection (Renwick & Chew, 1994).

Insects search for potential host plants using their visual and/or olfactory senses (Ramaswamy, 1988; Renwick & Chew, 1994). Some species are attracted to the shape of the plant's leaf (Städler,1974; Kostal & Finch, 1994), while others prefer plants of a specific shape (Ramaswamy, 1994). At close proximity to a host plant, insects will orient by using olfactory cues (Ramaswamy, 1988; Renwick & Chew, 1994; Honda, 1995). Host seeking behaviour and the frequency of landings also increase in response to favourable plant volatiles. Host-plant volatiles increased alighting in *Trichoplusia ni* (Landolt, 1989) and *Papilio polyxenes* (Feeny *et al.*, 1989).

Though vision and olfaction are important in identifying potential hosts, tactile stimuli also appear to have a significant influence on the process of selection (Städler, 1986; Renwick & Chew, 1994). Physical characteristics of the plant's surface are detected by non-porous mechanoreceptive sensilla, while the chemical character of a plant is detected by porous chemosensilla (Schoonhoven *et al.*, 1992). Both types of sensilla are found on the tarsi and/or ovipositor of insects. The mechanosensory sensilla provide the insect with valuable information about the plant substrate and its relative position on the plant. They also serve to release the drive for oviposition and control the timing action of the genital organs. Several studies have been done on lepidopterans to determine the types of sense organs that are involved in substrate evaluation. Such studies have characterized morphologically different sensilla of the females that may provide the insect with input about the nature of the host plant. Females of *Chilo partellus* have mechanosensory and

chemosensory hairs on the surface of their bi-lobed ovipositor (Waladde, 1983). Each mechanosensory or tactile hair contains a single neuron whose dendrite terminates at the base of the hair. Similar tactile hairs on the ovipositor have been described in *Spodoptera littoralis* (Chadha & Roome, 1980), *H. virescens* and *H. subflexa* (Baker & Ramaswamy, 1990). Lepidopterans whose ovipositors contain both mechanosensory and chemosensory hairs include *Phthorimaea operculella* (Valencia & Rice, 1982), *Ephestia kuehniella* (Anderson & Hallberg, 1990), and *Homoeosoma nebulella* (Reymonet & Faucheux, 1991). Other insects that have both taste and tactile hairs on their ovipositor are *Musca autumnalis* (Hooper *et al.,* 1972), *Locusta migratoria* (Rice & McRae, 1976), *Atherigona soccata* (Ogwaro & Kokwaro, 1981), *Lucilia cuprina* (Merritt & Rice, 1984)), *Urophora affinis* (Zacharuk *et al.,* 1986), and *Rhagoletis pomonella* (Stoffolano & Yin, 1987).

Contact chemoreception of surface chemicals by taste sensilla on the ovipositor, proboscis, or tarsi of lepidopterans is believed to be one of the most important steps in host recognition (Ramaswamy, 1994). The plant surface contains an abundance of stimulants and deterrents. Chemical recognition and subsequent acceptance or rejection of potential host plants is believed to occur through evaluation of such surface chemicals. The major class of compounds believed to be involved in feeding behaviour and ovipositional preference are the secondary plant metabolites including glucosinolates, isothiocyanates, terpenes, glycosides, and alkaloids. Certain alcohols, alkanes, carboxylic acids, esters, salts, sugars, and amino acids can also alter the insect's visiting behaviour. Several studies have shown that insects prefer polar and/or non-polar solutions of plant extract (Renwick & Radke, 1983; Nishida & Fukami, 1989; Honda, 1990). In all cases, females showed the highest preference to the complete extract while single compounds were not attractive. A preference was also shown towards a mixture of the chemicals, but the overall attractive effect of the mixture was still lower than the complete fraction.

The spruce budworm, *Choristoneura fumiferana*, is an oligophagous insect and a major pest of coniferous trees in North America. Host seeking behaviour in this insect is

affected by several factors, one of them being mating status (Rivet & Albert, 1990). Many other studies also suggest that mated females behave differently from virgins (Angioy *et al.*, 1983; Landolt, 1989; Yeh & Klowden, 1990; Bowen, 1991; Judd & Borden, 1992). In all cases, the act of mating caused behavioural changes that resulted in gravid females selecting the proper oviposition medium or laying eggs on the optimum food source.

In certain species mating is necessary before oviposition behaviour is initiated. Others, such as the spruce budworm, emerge with a full complement of mature unfertilized eggs, and following fertilization many females will actively seek suitable host plants for their future offspring. Städler (1974) found that substrates coated with the terpenes D-α-pinene and L-β- pinene stimulate oviposition by *C. fumiferana*. His results showed that gravid females oviposited on substrates treated with these compounds four times more often than on untreated twigs (controls). In another experiment, he demonstrated that untreated twigs of the balsam fir hosts were preferred to twigs coated with paraffin or washed with petroleum-ether. He also found that there were no significant attractive differences between an ether extract and a steam distillate of balsam fir needles. Thus, it can be concluded that the attractants and ovipositional stimulants are located on the surface of the leaf. Rivet and Albert (1990) showed that mated spruce budworm females are more selective than their virgin counterparts. They found that mated females lay three times more eggs on untreated twigs compared to twigs in which the wax layer is removed. Results from this study indicate that the stimulatory compounds that encourage oviposition are found on the surface of the leaf. The Rivet and Albert study also showed that amputation of the proboscis resulted in loss of host discrimination capability. Städler (1974) observed that the antennae and proboscis are important organs in host recognition. Ablation of both greatly reduced the number of eggs laid on balsam fir.

Juvenile spruce budworm survival is entirely dependent on whether or not the larvae have access to appropriate food sources during the course of their development. Their chance of survival is also directly related to the adult female's ability to find the

correct host plant. Mating can induce changes in the female's peripheral sensory neurons. These changes in sensitivity of the sensilla could presumably result in mated females displaying the proper behavioural response.

The goal of this study is to use electrophysiological techniques in an attempt to explain the observed behavioural differences between mated and unmated spruce budworm females in their host plant selection for oviposition. The major objectives are the following:

1. Describe the types of mechano- and chemosensilla on the ovipositor of *C. fumiferana*, and determine their innervation.

2. Rivet and Albert (1990) have shown that spruce budworm females lose their ability to discriminate host leaves devoid of surface waxes. The surface extracts they used may have also contained some polar compounds. Derridj *et al.* (1989) found that certain polar compounds also stimulate oviposition. If the ovipositor of *C. fumiferana* females contains chemosensory hairs, I would like to obtain physiological recordings from these sensilla to compounds suspected to be used by the insect in host selection. These compounds include surface waxes, the secondary alcohol found in most conifers and the highest individual surface component of white spruce waxes (nonacosanol), and polar compounds (in mixtures and individually) from white spruce needles. This data would constitute the first set of recordings obtained from taste sensilla on the ovipositor of this insect.

3. Mated and unmated insects behave differently (Landolt, 1989; Yeh & Klowden, 1990; Bowen, 1991; and Judd & Borden, 1992). I will compare results between mated and unmated insects to determine if there are any differences in their response patterns. I can then assess whether any of the physiological differences can account for previously observed behavioural differences between mated and unmated females by Rivet and Albert (1990).

# MATERIALS AND METHODS

*Insects*

Spruce budworm larvae were obtained from the Great Lakes Forest Research Center (Sault Ste. Marie, Ontario) as diapausing second-instar larvae. They were reared on an artificial diet (Grisdale, 1984) in an incubator with a 16L:8D photoperiod, 60% humidity and an ambient temperature of 22°C. After a successive number of moults, the 5th or 6th instar larvae were sexed, separated, and kept in the incubator until pupation. The separated male and female pupae were placed in wooden mesh cages (35x35x20 cm, LxWxH). Both sexes were given free access to water and a 5% sucrose solution. Tests were performed on both virgin and mated females (2-6 days old). Unmated insects were obtained by placing only female pupae in a mesh cage. The pupae were checked daily and cages with emerging insects that were not required for testing were discarded. The test insects were obtained by placing approximately 10 females (less than 1 day old) with an equal number of males (2-3 days old) in the same mesh cage. Pairs in copula were separated and placed in round plastic jars until mating was complete. The following day the male was discarded and females were tested after they had laid their clutch of eggs. If the eggs hatched into larvae, the physiological recordings obtained from that female were put into the pool of mated results for a given experiment.

*Foliage*

Females normally oviposit from late June to July. White spruce branches used in this study were collected in August 1993 at the Morgan arboretum in Ste. Anne de Bellevue, Quebec. Samples were sealed in plastic bags and kept at -20°C until needed.

*Wax Extraction*

Spruce needles were soaked in hexane for 30 seconds (Maloney *et al.*, 1988).

Exposed twig ends were kept out of the hexane to avoid leaching of internal chemicals into the extract. Epicuticular waxes were recovered by evaporating the hexane in a gentle stream of cotton filtered nitrogen gas. The extracted material was dissolved in a mixture of 2.5 ml tetrahydrofuran (THF), 7.5 ml 133 of mM/l potassium chloride (KCl) and 0.1 ml of tween (Städler & Buser, 1984). This solution is referred to as 1X wax extract. Dilutions (0.1X and 0.01X wax) were made by taking aliquots of the 1X wax solution and diluting it with a stock solution of 25:75 THF/KCl.

*Water Extraction*

Spruce needles were allowed to sit in 10 ml of distilled water for a period of 1 or 2 hours. Upon removal of the needles, a known quantity of KCl crystals was added to the water to make the final solution 100 mM/l.

*Polar Solutions*

A mixture of the 14 amino acids found in the highest proportions in white spruce needles (Albert & Parisella, 1988) was dissolved in 50 mM/l KCl (1X). A 0.1X amino acid solution, 25 mM/l sucrose in 50 mM/l KCl, and 50 mM/l proline in 50 mM/l KCl were also used. Nonacosanol, the highest individual chemical component of spruce needle surface waxes, was also used as a test chemical in both polar (100 mM/l KCl) and non-polar (0.1X 25:75 THF/KCl) solvents (Tulloch, 1987).

*Electrophysiology*

Female spruce budworm moths were immobilized by placing them at $0°$ C for 5-10 minutes. The distal portion of the abdomen with the ovipositor was removed from the rest of the body and mounted on a glass microelectrode filled with a saline solution (150 mM/l KCl + 10 mM/l NaCl). The test and control solutions were applied in random order to the tip of the ovipositor sensilla. A 3-5 minute recovery period was allowed between

successive applications the solutions. The recordings were made with a DC amplifier, stored onto digital audio tape, and digitized at 10,000 Hz for a one second period by using the *Sapid Tools* computer program (Smith *et al.*, 1990).

Eight to twenty-four female insects were used per experiment, with each ovipositor being used only once per experiment. Degrees of freedom in experiments 1, 2, 4, and 5 represent (n-2) number of hairs tested. For experiments 3 and 6, (n-1) hairs were tested. Approximately 2-4 hairs/insect were tested with all the solutions for a given experiment.

Recordings were printed in three 333 millisecond segments. Individual spikes were counted and classified into one of four types. Classification of neuronal cell types of a sensillum was based on characteristics such as spike width and amplitude, and peak to trough distances of the observed waveforms. Nomenclature of the four cellls in this study is consistent with that of Schnuch and Hansen (1990). The error rate for manually scoring the tracings was determined by twice tabulating firing rates from the same 14 recordings. Fifty-six comparisons were made and the mean percent difference for cells 1 to 4 were 4.15, 10.17, 18.03, and 9.27%, respectively.

*Morphology- Staining*

Female ovipositors were stained by a method for staining insect nervous tissue (Zacharuk, 1962). Newly emerged adult female spruce budworm were trapped and anesthetized at $3^0$C. The abdomens were injected with a solution of 0.4 % methylene blue : 0.6 % NaCl solution. After a minimum of two hours, the ovipositors were excised and fixed in a saturated solution of ammonium molybdate. The ovipositors were thoroughly washed in distilled water, dehydrated in a graded series of alcohols, and cleared in xylol. They were mounted on slides in permount and photographed.

*Scanning electron microscopy (SEM)*

For SEM studies, cleaning of the ovipositors was essential because the sensillar

surfaces were covered with debris. Several cleaning methods were used (Baker & Ramaswamy, 1990; Klijnstra & Roessingh, 1986; Stoffolano & Yin, 1987; Valencia & Rice, 1982), but only two yielded clean specimens (Cuperus, 1985; Kapoor, 1989). The excised ovipositors were immersed in physiological saline at $60^0$C for 15 minutes, washed twice in this same solution and sonicated for 30 seconds. The specimens were washed in warm 10% acetic acid in saline, rewashed three times in warm saline, once in 5% KOH for 10 minutes, twice in warm saline again, and finally fixed in buffered 2.5% glutaraldehyde followed by 1% osmium tetroxide (Kapoor, 1989). They were dehydrated in an ethanol series, critical point dried, glued to stubs, and coated with about 100 Å of gold in a sputter coater. In another method, excised specimens were immersed in boiling tetrachloromethane and rewashed 4-5 times in fresh stock of the same solution, air dried and coated with gold (Cuperus, 1985). About 40 ovipositors were analyzed, but only 14 were used to record physical attributes of sensilla. Specimens were examined and photographed in a Hitachi S-520 SEM.

*Transmission electron microscopy (TEM)*

This work was done at Institut Armand Frappier, Laval, Quebec. Unstained ovipositors were fixed in buffered gluteraldehyde, followed by osmium, dehydrated in graded ethanol-propylene oxide, and embedded in Epon-812 mixture. The blocks were trimmed and ultra-thin sections were cut with an LKB ultramicrotome. The sections were stained in a 5% uranyl acetate : 50% ethanol solution for 20 minutes in complete darkness, thoroughly washed in distilled water and placed in lead citrate for 5 minutes. After washing twice in water, air dried grids were viewed and photographed with a Hitachi H-7100 TEM.

# RESULTS

*Morphology*

The ovipositor of the female spruce budworm, *C. fumiferana*, is a bi-lobed kidney shaped structure (Figure 1) bearing an array of sensilla (Figure 2). Four morphologically distinct types of trichoid sensilla are identified (Table 1).

Table 1. Morphological differences between sensilla trichodea on the ovipositor of the spruce budworm (ranges in parentheses). Sample sizes (n) represent hair types from 14 ovipositors.

| Characters | Type I (n=8) | Type II (17) | Type III (8) | Type IV (9) |
|---|---|---|---|---|
| Length (μm) | 33.6 (17.6-48.1) | 62.5 (37.2-96.1) | 124.1 (103.8-173.5) | 131.2 (65.0-202.8) |
| Basal diameter (μm) | 4.4 (3.3-5.7) | 3.2 (2.5-4.4) | 4.2 (3.8-4.6) | 4.0 (2.5-4.8) |
| Pore to pore distance (μm) | n/a | 0.7 (0.2-1.5) | 1.1 (0.3-1.7) | n/a |

Type I sensilla are short and found throughout the ovipositor. They are stout at the base and taper quickly. The tapered end of the sensillum is thin and generally bent, probably due to processing in preparation for microscopy. Their surface is longitudinally ribbed and the ridges bear a row of pores (Figure 3).

Type II sensilla are of medium length. They are also unique because they posses characteristics of both olfactory (multiporous) and taste (uniporous) sensilla. They have a multiporous ribbed shaft (Figure 4), and narrow to a blunt uniporous tip (Figure 3). Although few are found throughout the ovipositor, they are clustered centrally on its

broadest portion. Specimens stained with methylene blue (Figure 5, arrowhead and Figure 6) show an aggregation of 4 cell bodies in a region close to a sensillum. The TEM study also shows that there are at least 4 chemosensory neurons whose dendrites extend into the shaft of each sensillum (Figure 7).

Type III sensilla are long multiporous hairs (Figure 3). They are found predominantly along the periphery of the ovipositor, but are also interspersed among other hair types. These sensilla have about 13 ridges and they taper very gradually. Rows of pores on the shaft of the hair are located on and between the ridges and the apical 2-3 μ portion of these hairs is ridgeless. Methylene blue preparations show that there is only one dendrite extending into their shaft (Figure 5, arrows).

Type IV sensilla are non-porous long hairs. They are the longest of the four hair types, and are probably mechanoreceptors . They are the only smooth trichoid hairs on the ovipositor (Figures 2 and 3). Their distribution pattern is similar to the Type III multiporous hairs.

*Physiology*

All physiological experiments were done on Type II sensilla because they are the only hairs with the structural characteristics of known gustatory chemosensilla found on the ovipositor of *C. fumiferana*. Type I and III hairs are strictly multiporous and probably serve an olfactory function (Faucheux, 1991). Type IV hairs have no pores and a single dendrite terminates at their base. They probably serve a tactile function. Individual physiological tracings were printed using the *Sapid Tools* computer program (Smith *et al.*, 1990) and analyzed visually. Based on the responses obtained, up to four cells responded to a given solution. Cell 1 (sugar cell) exhibits a large amplitude monopolar waveform and responds preferentially to solutions of sugar (Figure 8, a). Cell 2 (salt 1 cell) is characterized by a fast biphasic spike (Figure 8, b). The third cell (salt 2 cell) is distinguishable from Cell 2 because it is a slower, larger biphasic spike (Figure 8, c). Cells

2 and 3 are both classified as salt cells because they respond best to pure salt solutions, their firing rates are reduced upon the addition of other solutes, and they are distinguished by a bipolar wave form (Schnuch & Hansen, 1990; Schoonhoven *et al.*, 1992; Wolbarsht, 1965). Cell 4 (water cell, not shown in Fig. 8) has a low amplitude, slow monopolar spike. It generally fires at a low rate in all cases.

For all experiments involving comparisons between mated and unmated insects, a one-way analysis of variance (ANOVA) was used to determine if the mating status of the insect had any effect on its physiological response. To control for comparisons between mated and unmated insects to more than one stimulus, a Bonferroni correction was applied to alpha (0.05). Only results whose $P$-values were less than the corrected alpha critical ($\alpha'$) were considered statistically significant. The $\alpha'$ depends on the number of comparisons in a given experiment (4-16) and varied from 0.0125 to 0.003125.

*Experiment 1: Wax & THF/KCl*

Wax at an average concentration of 0.24 mg/leaf dissolved in 10 ml of THF/KCl was tested against the solvent (control). Figure 9 shows that there are 3 cells firing in mated and unmated insects. Cell 4 also responds in mated insects. It fired at a significantly higher rate in mated compared to unmated insects for the control solution ($\alpha'= 0.00625$, Figure 9). There were no significant differences for any other cell types for both solutions (Figure 9). No significant differences between the wax and control solutions were found within each mating group for any of the cell types.

*Experiment 2: Reduced concentrations of Wax & THF/KCl*

It would be difficult to determine an exact wax concentration per unit surface area that the insect uses to evaluate a potential host. Since the responses to a full strength wax and control salt solution were quite low, tests were done using 0.1X, 0.05X, and 0.01X wax solutions and a 0.1X control solution. Less concentrated solutions were used because

the original 1X wax solution appeared very cloudy, and was probably super-saturated and above the physiological response threshold of the ovipositor taste sensilla. Analysis of the firing rates in response to the lower concentrated test materials showed that there was a noticeable increase in the average response to any given solution (20-30 impulses/s as compared to 10-20 impulses/s). Figure 8 shows a typical response of Type II sensory hairs to the 0.01X wax concentration. At $\alpha$'= 0.00325, there were no significant differences between mated and unmated females in response to the control and all concentrations of the wax solutions (Figure 10).

*Experiment 3: Nonacosanol & THF/KCl*

Figure 11 shows the average firing frequency of Cells 1-4 for mated females only. Results from a two-sample t-test show that there were no significant differences ($\alpha$'= 0.0125) in the firing rates of Cells 1-4 to the control and nonacosanol solutions ($df_{1,19}$: $P$=0.67, 0.04, 0.97, and 0.63, respectively).

*Experiment 4: Water Extracts*

This test was done to determine whether the Type II sensilla on the ovipositor are sensitive to any polar compounds that may have leached out to the plant's surface. A one- and two-hour water extract, nonacosanol in a polar solvent, and a control solution of KCl were used in this experiment. Both mated and unmated insects showed consistent response patterns to all 4 test stimuli (Figure 12). There were no differences in firing rates of the 3 cells between mated and unmated females in response to 100 mM/l KCl, the 1- and 2-hour water extract ($\alpha$'= 0.004). The response of Cell 1 to nonacosanol was significantly lower in mated compared to unmated females (Figure 12).

*Experiment 5: Polar Compounds*

Results from stimulation of Type II sensilla with sucrose, proline, and two

concentrations of amino acids found in white spruce are presented in Figure 13. Cell 1 is the most active cell of the four types for both unmated and mated females for virtually all the test solutions. In mated insects it has a significantly elevated firing rate for sucrose and proline ($\alpha'= 0.003125$, Figure 13). Cell 2 in response to 0.1X amino acids and Cell 3 in response to sucrose both respond significantly more in mated than unmated females.

*Experiment 6: Male extract*

Several male copulatory organs were washed in hexane, redissolved in 0.1X THF/KCl, and subsequently tested on the chemosensory hairs of unmated female ovipositors. Comparisons were made against a control solution of 0.1X THF/KCl. Figure 14 shows the mean response of the 4 cells to both the control and male extract solutions. Cell 1 was frequently the only cell firing in response to the male extract solution, and at times it responded up to 100 impulses/s. Results from a two-sample t-test indicate that there is a significant difference between the mean firing rate of Cell 1 ($\alpha'= 0.0125$, Figure 14) in response to the two solutions.

*Total response to all solutions*

Total sensory input is critical to the insect (Calvert & Hanson, 1983). Figures 15 and 16 represent the total mean response of the 4 cells to all control and test solutions used in this study. Analysis of variance between mated and unmated insects shows that mated insects respond significantly higher to solutions of sucrose ($df_{1,25}$; F=25.23; P<0.001), proline ($df_{1,26}$; F=33.42; P<0.001), 0.1X amino acids ($df_{1,29}$; F=14.82; P<0.001), 1X amino acids ($df_{1,19}$; F=16.36; P<0.001), and 0.01X wax ($df_{1,19}$; F=6.10; P=0.02). In response to nonacosanol in a polar solvent, mated insects responded at a lower rate than unmated females ($df_{1,36}$; F=5.99; P=0.02).

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Figure 1.  Scanning electron micrograph (SEM) showing two lobes of the ovipositor with trichoid sensilla throughout the surface.  Magnification X80.

Figure 2.  SEM showing more detail of the distribution of trichoid sensilla on a portion of one lobe of the ovipositor.  Magnification X350.

Figure 3.  SEM showing the four types (I, II, III, IV) of trichoid sensilla found on the ovipositor of female *C. fumiferana*.  Magnification X1,100.

Figure 4.  SEM of Type II sensillum showing the multiporous shaft; arrowheads point to individual pores.  Magnification X6,000.
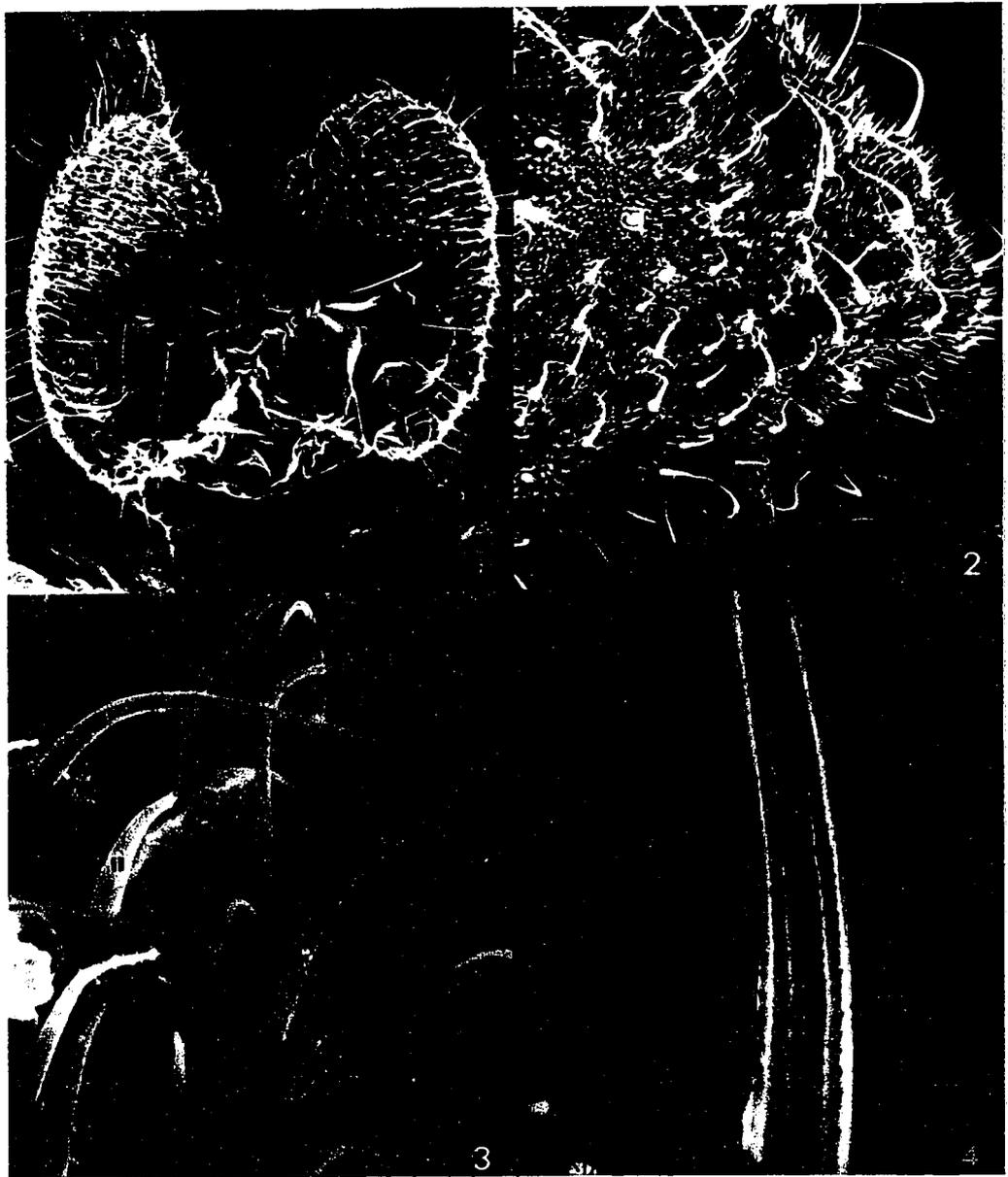
Figure 5. Wholemount of ovipositor lobe stained with methylene blue. Arrows

point to cell bodies of single neurons innervating Type III mechanoreceptor

sensilla. Arrowhead points to cell bodies of a group of four neurons innervating a

Type II sensillum used to obtain electrophysiological responses in this study.

Magnification X540.

Figure 6. Enlargement of Figure 5 showing cell bodies of chemosensory (1, 2, 3,

4) and mechanosensory neurons (5) innervating a Type II sensillum. Magnification

X1,000.

5



6

Figure 7. Transmission electron micrograph showing dendritic sheaths
(arrowheads) surrounding the dendrites of four chemosensory neurons (1, 2, 3, 4)
innervating a Type II sensillum. Magnification approx. X25,000.

0.01X Wax
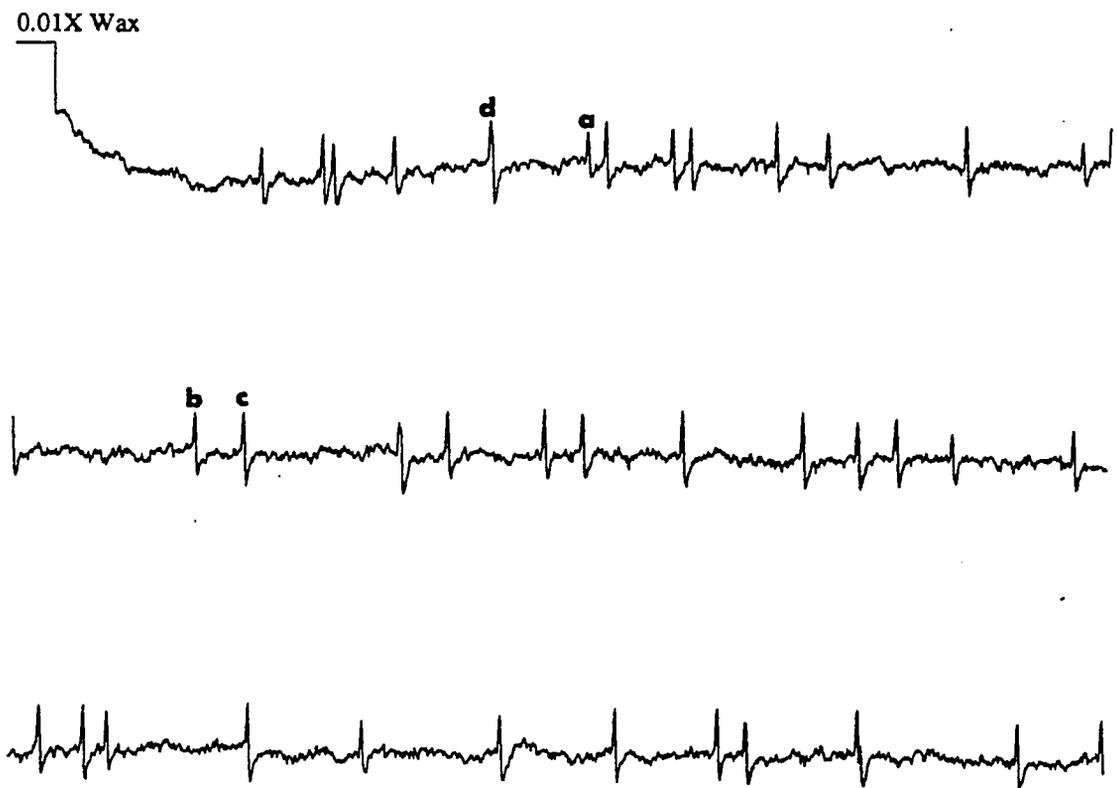


Figure 8. Electrophysiological traces showing responses from a Type II sensillum to stimulation with 0.10X Wax extract. Entire trace is 1 s and is divided into three 333 msec segments. a, Cell 1 (sugar cell); b, Cell 2(salt 1 cell); c, Cell 3 (salt 2 cell); d, 'double' spike.
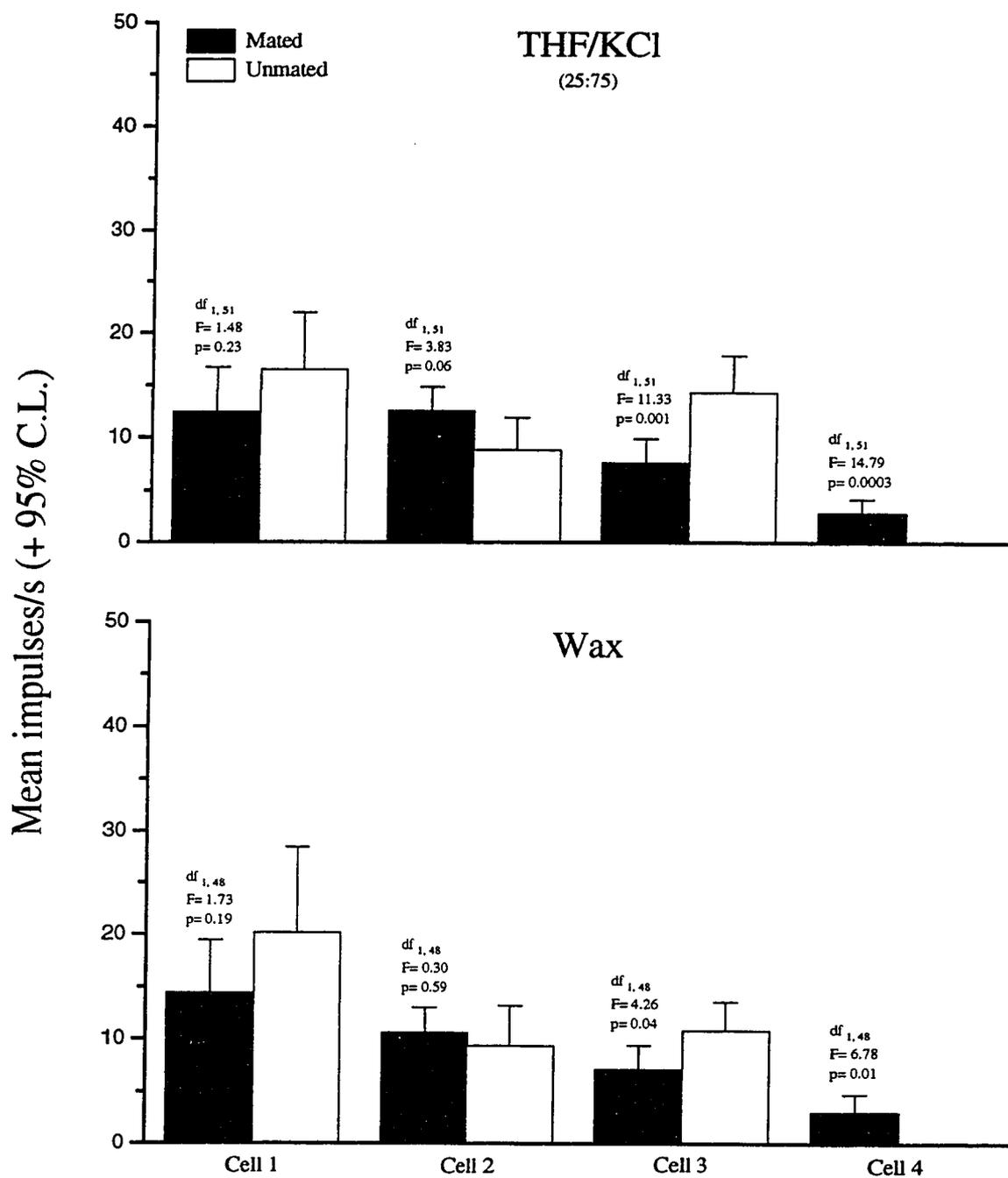
Figure 9. Mean response of Type II sensilla to 1X wax extract in a 25:75 THF/KCl solution and a control solution of THF/KCl. ANOVA results are also presented for comparisons between mated and unmated insects.
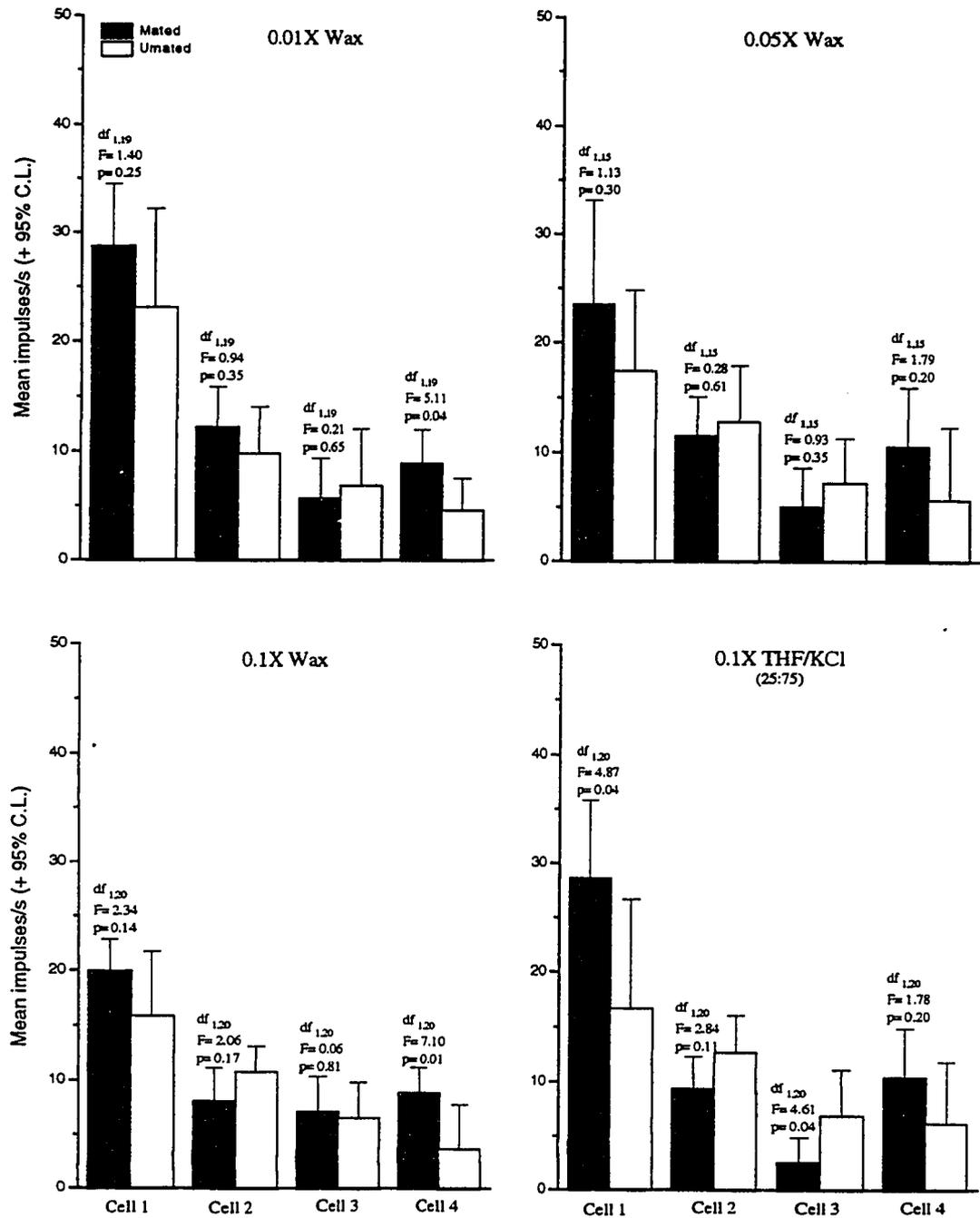
Figure 10. Mean responses of Type II sensilla to reduced concentrations of waxes (0.1X, 0.05X, and 0.01X) all dissolved in 0.1X THF/KCl (25:75). ANOVA results are also presented for comparisons between mated and unmated insects.
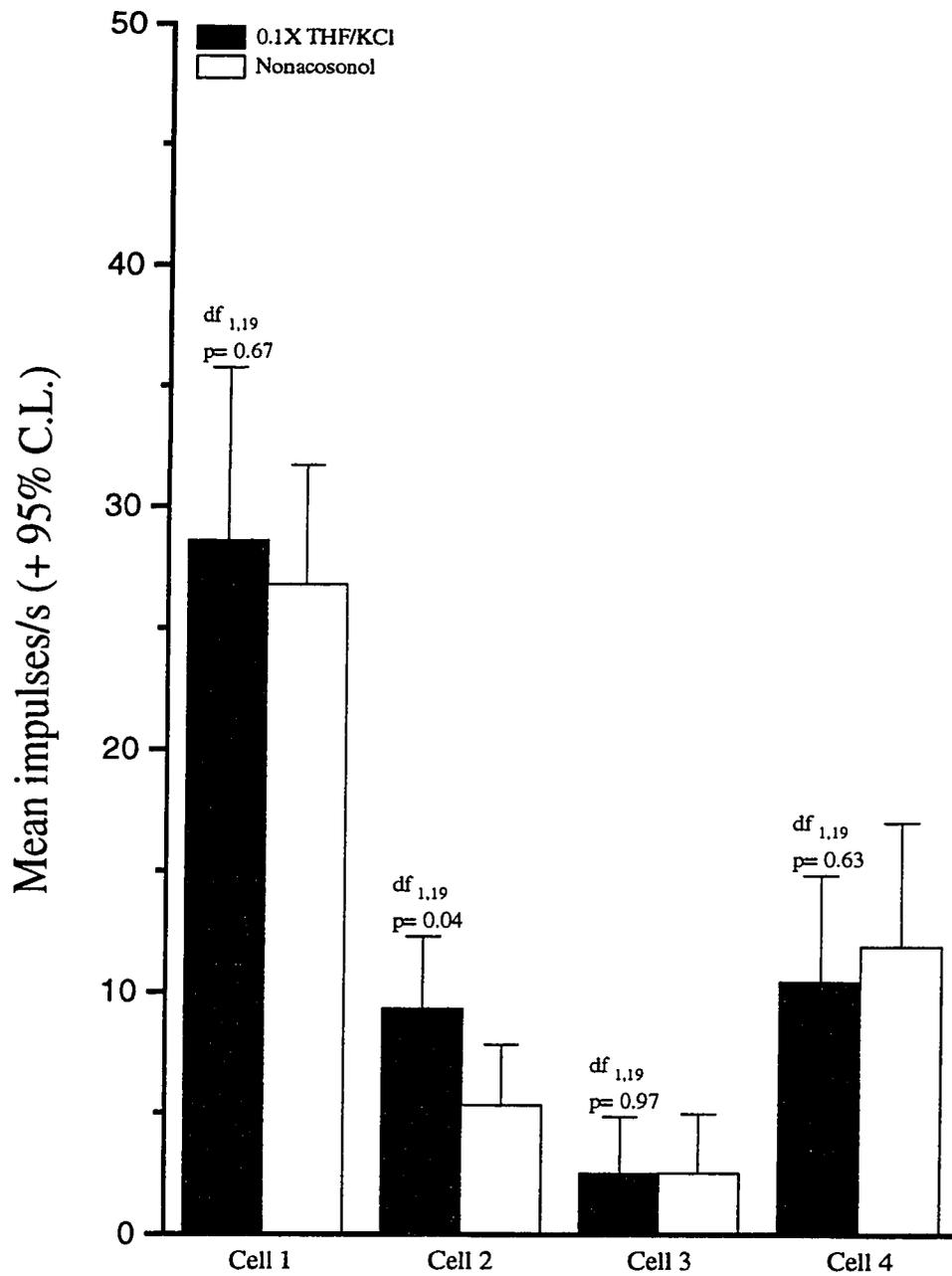
Figure 11. Mean responses of Type II sensilla of only mated insects to Nonacosanol in a non-polar solvent, and a control solution of THF/KCl.
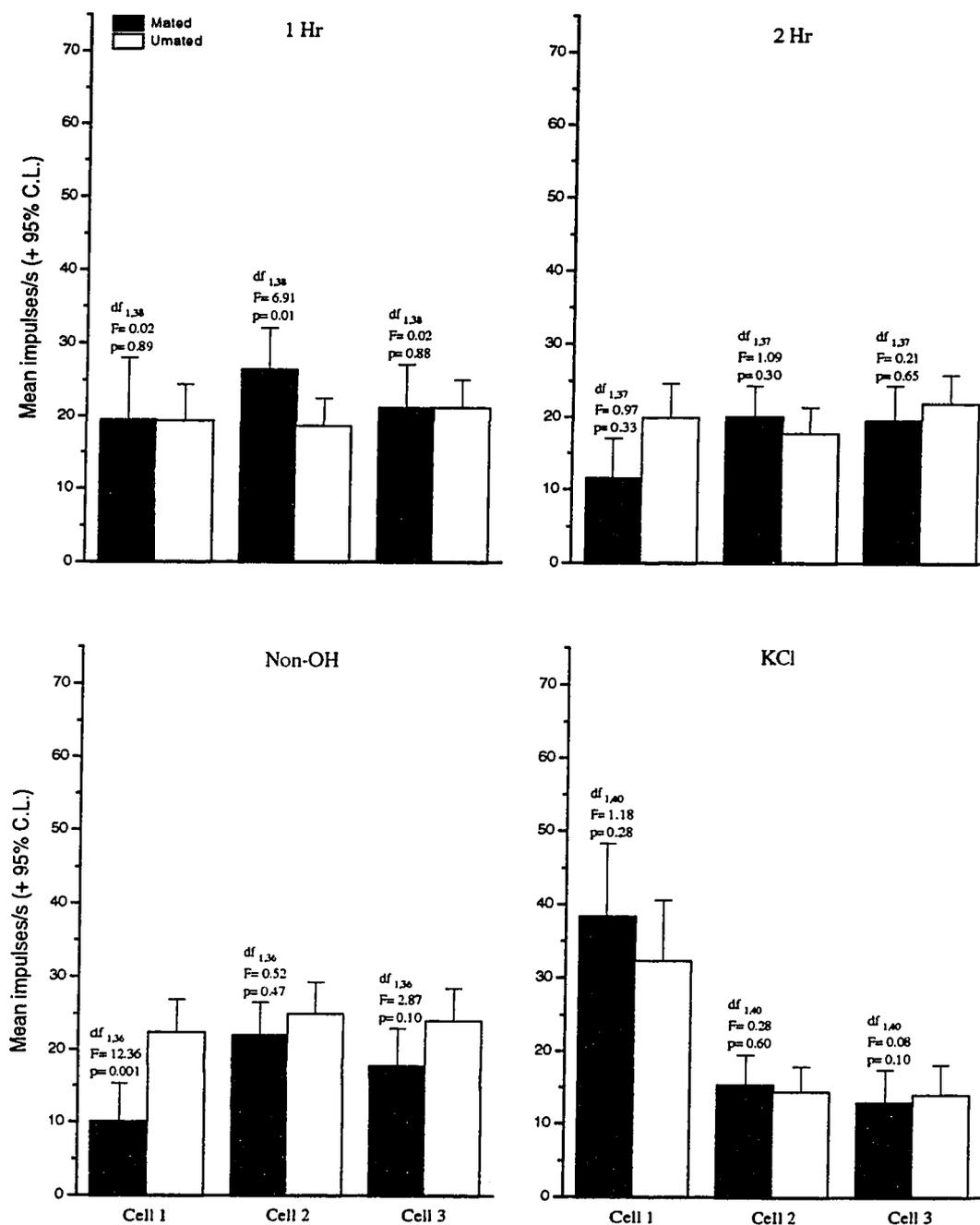
Figure 12. Mean response of Type II sensilla of mated and unmated insects to solutions of 100 mM/l KCl, one and two hour water extracts in KCl and Nonacosanol in KCl. ANOVA results are also presented.
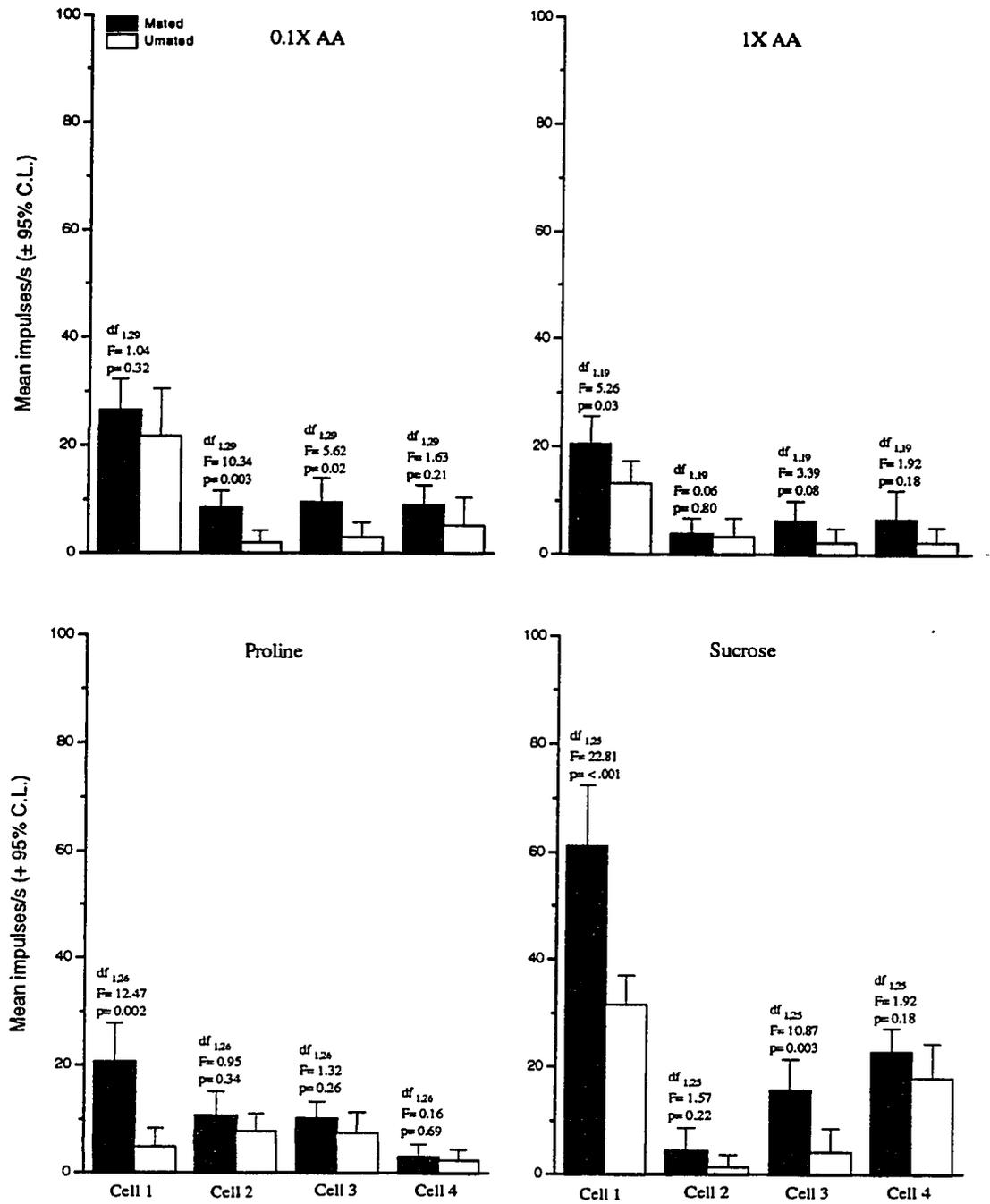
Figure 13. Mean responses for mated and unmated insects to polar nutrients
including sucrose, proline, and two concentrations of a mixture of amino acids.
All test substances are dissolved in 50 mM/l KCl. ANOVA results are also presented.
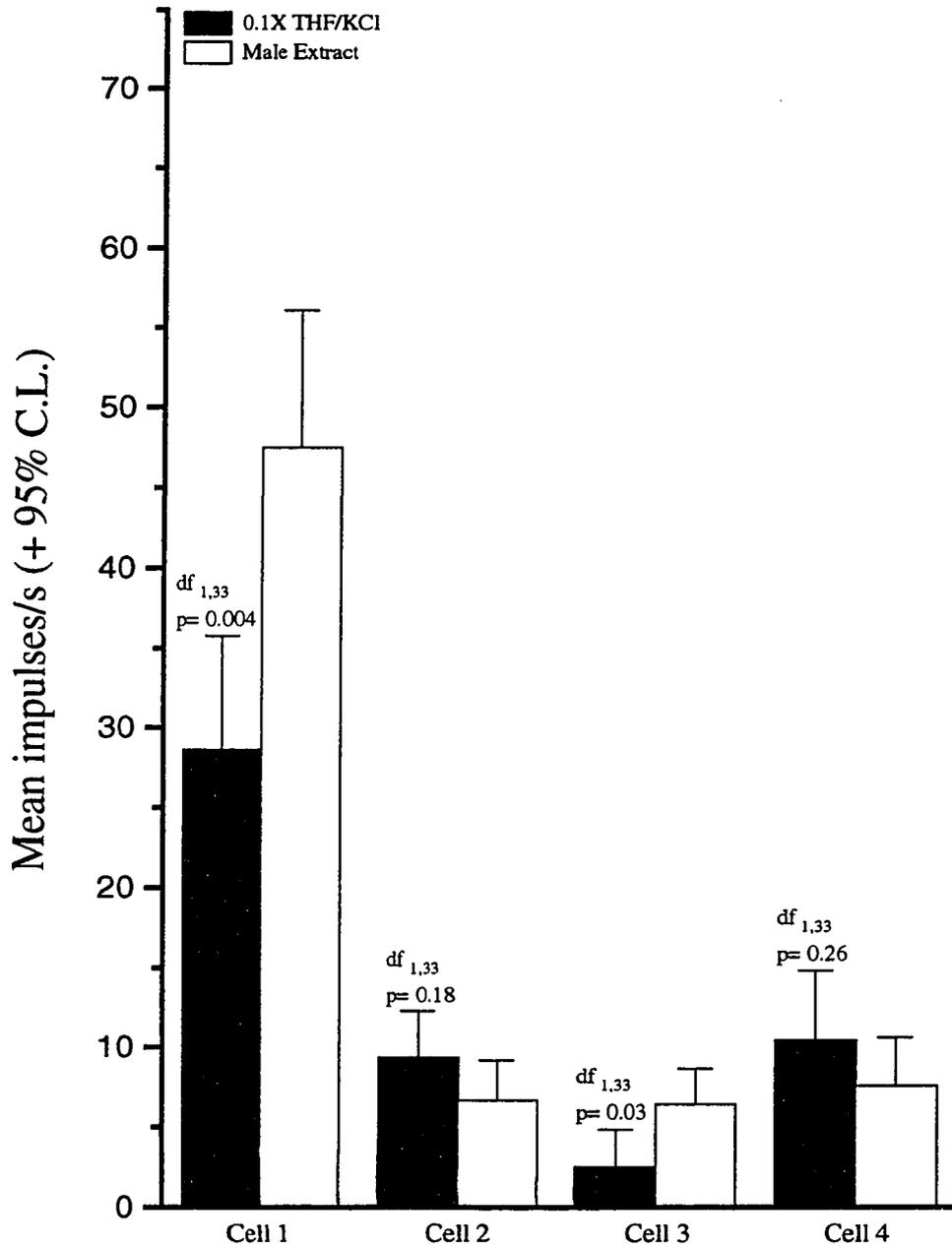
Figure 14. Mean responses of ovipositor sensilla of only unmated females
to a control solution of 0.1X THF/KCl and a hexane wash of the male copulatory
organs dissolved in a similar solution.
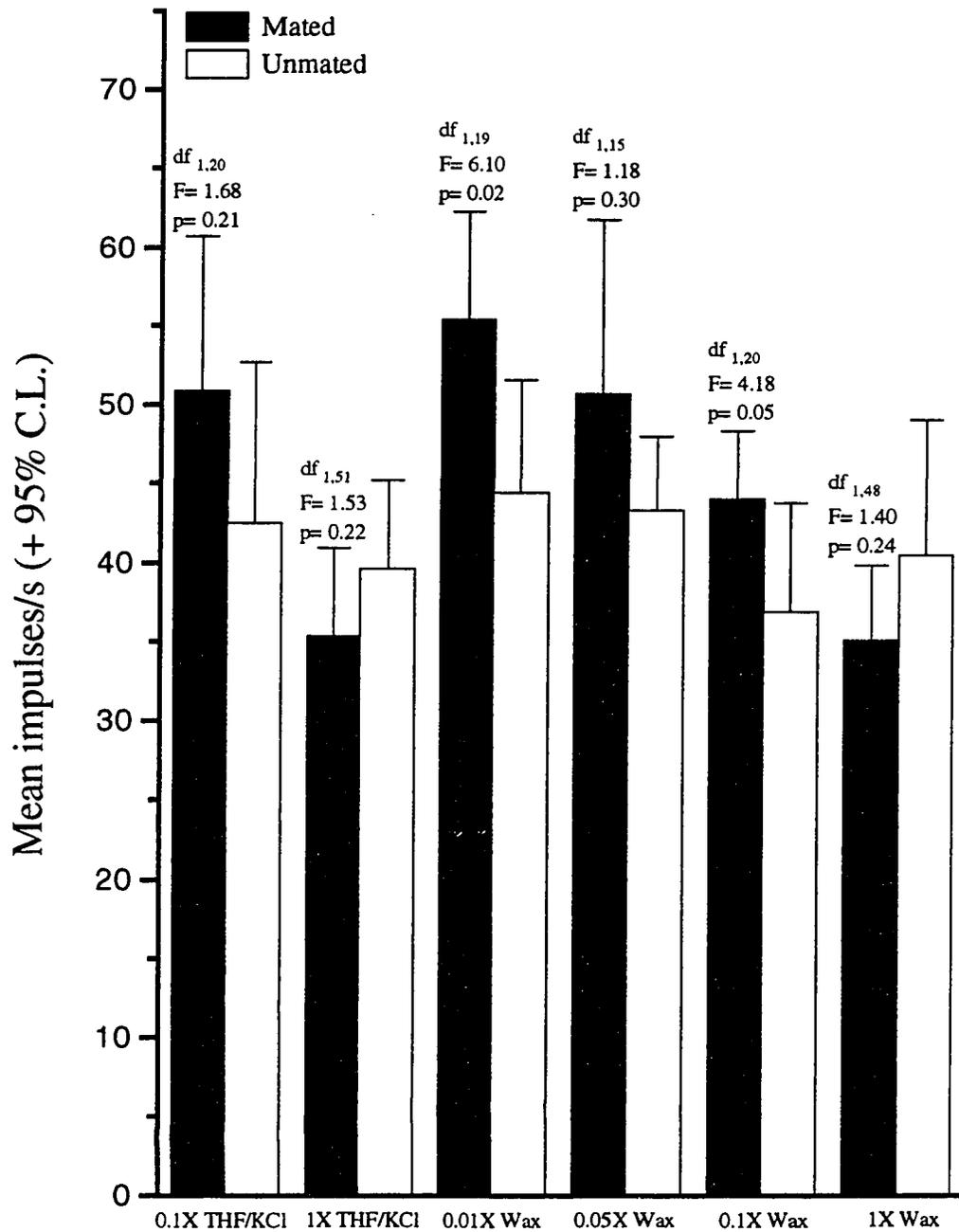
Figure 15. Mean responses of mated and unmated insects to all concentrations of non-polar control and wax solutions used in our experiments. Each bar represents the sum total response from the four neurons present in each sensillum.
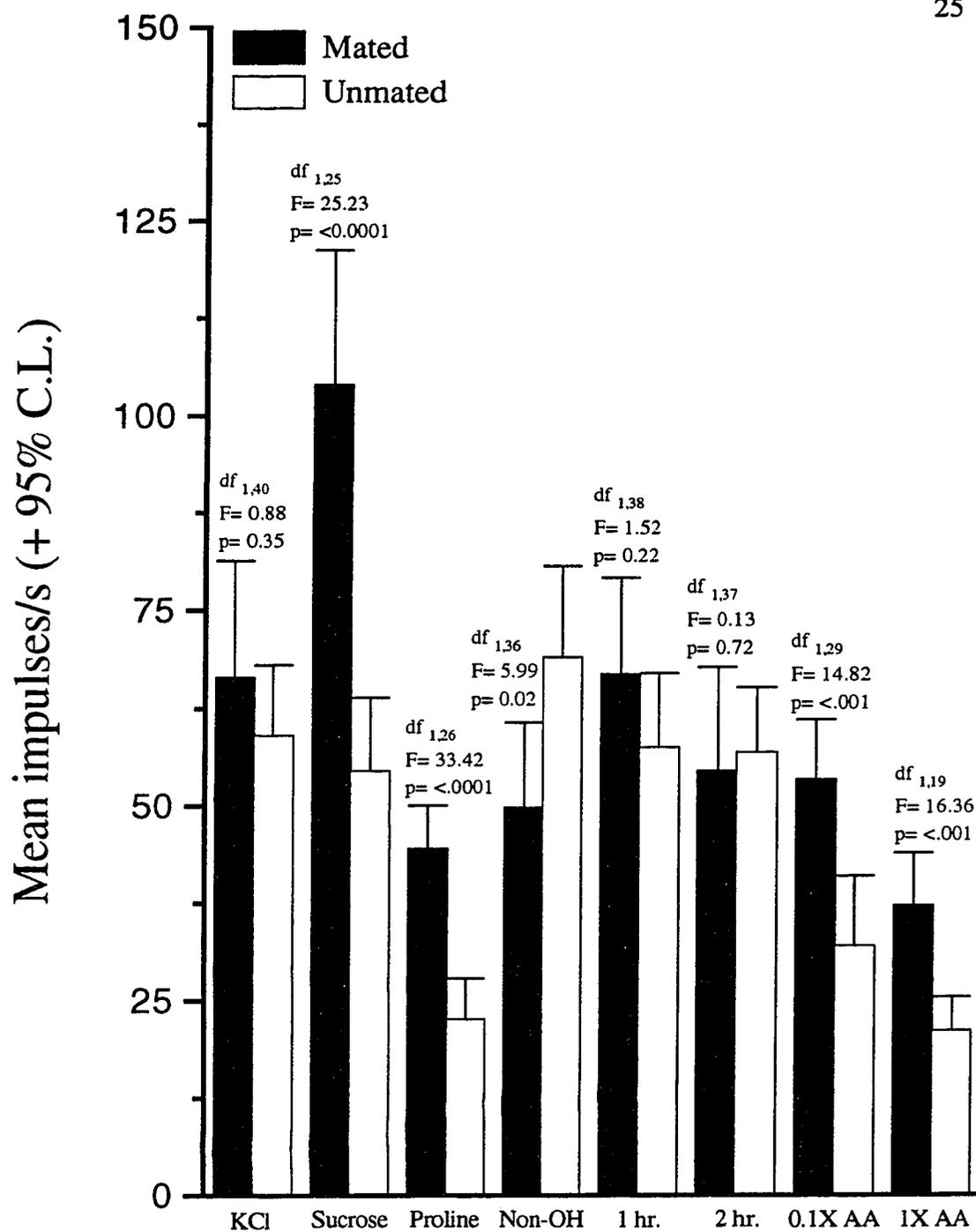
Figure 16. Mean respones of mated and unmated insects to all polar solutions used in our study. Each bar represents the summated response from all four sensory cells in each sensillum.

# DISCUSSION

This first morphological investigation of the female *C. fumiferana* ovipositor shows that it bears both mechanosensory and chemosensory hairs. The presence of long and short multiporous sensilla and long aporous mechanosensory hairs adds to the growing list of insect species whose ovipositors possess sensory structures used in host discrimination (Anderson & Hallberg, 1990; Faucheux, 1991; Reymonet & Faucheux, 1991; Valenia & Rice, 1982). More importantly, a multiporous Type II sensillum with a pore at the tip was also found on the ovipositor. There are few reports in the literature of any lepidopteran species having such a hair type. Faucheux (1988) found a similar basiconic sensillum on the ovipositor of *Monopis crocicapitella*. He postulated that it serves an olfactory function. In this study, responses from the Type II hairs indicate they react in a manner which is similar to most uniporous taste hairs; however, whether they also serve an olfactory function has yet to be determined.

The Type II hairs on the ovipositor of *C. fumiferana* responded to all test stimuli in my experiments. The size, shape, interaction, and temporal response of the neurons indicates these hairs respond in a typical manner of uniporous chemosensilla (Schoonhoven *et al.*, 1992). Although each hair contains 4 chemosensory cells, Cell 1 is usually the most active neuron. Cells 2-4 also respond to all solutions. This indicates that the dendrites of all 4 neuronal types (sugar, salt 1, salt 2, and water) contain a variety of receptor sites that result in the majority of cells firing to any given solution (Schoonhoven *et al.*, 1992; Wolbarsht, 1965). Differences in firing rates of the cells will allow the insect to discriminate between various solutions at either the level of the peripheral or central nervous system.

In this study, physiological responses to surface compounds and common nutrients were obtained only from Type II sensilla. My results indicate that these are not capable of discriminating between the wax and the control solutions. Based on the responses obtained, both mated and unmated females showed no significant differences in responses to the host epicuticular waxes or nonacosanol versus the control solution. Although the results were not

significant for the reduced concentrations of waxes, mated females did show an increased response from Cell 1. It is also interesting to note that with an increasing concentration of wax in solution, total response rate decreases for mated females. This implies that even at a concentration of 0.01X wax, perhaps the stimulative component of the waxes in solution may be out of the threshold range of the ovipositor sensilla. There is the possibility that the concentration of the waxes used was too strong. Nair and McEwen (1976) have shown that even strong stimulants such as isothiocyanates are repellent at high concentrations.

Using polar extracts of surface compounds, no differences were found between responses of mated and unmated insects to the one- and two-hour water extracts. This suggests that the female is not using a combination of water soluble surface chemicals to decide if she will oviposit. Since plant metabolites are known to leach onto the surface of the leaf, the composition of the waxes becomes variable over time. This change in wax chemistry probably reflects the physiological state of the host plant and can cause behavioural changes in the insect (Maloney *et al.*, 1988). Derridj *et al.* (1989) showed that the ovipositional preference of the European corn borer (ECB), *Ostrinia nubilalis*, was related more to the phenological stage rather than the species of plant. Preferences to the water extracts may have been seen had the foliage been collected during the egg laying period in late June or early July.

Renwick and Radke (1983, 1985) found that female *Pieris rapae* were deterred by non-polar extracts, but were attracted to hosts by water soluble compounds. The females of this species could also distinguish between various concentrations of the polar fractions. Studies have shown that sugars can be perceived in the ovipositional media (Rice & McRae, 1976; Eisemann & Rice, 1985). Hedin and McCarthy (1990) showed that sugars stimulated oviposition more often than any other class of compounds. Derridj *et al.* (1989) also found that ovipositional preference of ECB was highly correlated with the presence of fructose. This preference was further enhanced if glucose, sucrose, and proline were also on the leaf surface. The ovipositor sensilla of *C. fumiferana* females are capable of detecting sucrose solutions as are those on the proboscis (Städler & Seabrook, 1975) and tarsal sensilla (Mitchell &

Seabrook, 1974). This study also shows that there are significant differences between mated and virgin females in response to sucrose, proline, and amino acids. The mated females are more responsive to this compound, indicating that they could be using surface sugars as a possible host locating cue.

Certain amino acids have been shown to induce oviposition in *Trichogramma dendrolimi* (Zhi-Xin & Junde, 1982). Hedin *et al.* (1993) also showed that the southwestern corn borer larvae, *Diatraea grandiosell* Dyar, are more responsive to amino acids than they are to sugars. For these insects, a mixture of amino acids elicited a stronger response than a complete plant extract. Proline is a compound which is induced by a stress such as drought, and it could possibly be used by visiting *C. fumiferana* females to identify vulnerable spruce trees. Tisdale and Wagner (1991) showed that females of *Neodiprion fulviceps* were actually able to discriminate between stressed and non-stressed host plants. In response to proline, Cell 1 of Type II sensilla on the ovipositor fired at a significantly higher rate in mated females compared to virgins. The total response to proline was also higher in mated insects. These results suggest that mated females are more responsive to proline and this increased sensitivity may provide the insect with valuable information on the physiological state of the host tree. The results of this study also indicate that mated females are more sensitive to mixtures of amino acids than unmated insects. It is also interesting to note that the total response to a 0.1X amino acid mixture is higher than a 1X solution. This finding is consistent with the findings of the wax experiment, and again, perhaps the concentrations used are beyond the true physiological range of the ovipositor chemosensilla.

The insect brain integrates all positive and negative sensory input. This central processing ultimately allows the insect to make the appropriate decision (Dethier, 1982; Ramaswamy, 1988, 1994; Renwick & Chew, 1994) under a given set of circumstances. The majority of sensilla on the ovipositor of lepidopterans are mechanosensory. Physiological responses from these provide the insect's central nervous system with valuable information about the plant substrate. Both *Chilo partellus* and *Eldana saccharina* rely on their tactile

ovipositor sensilla in making decisions to deposit eggs (Waladde, 1983). *E. saccharina's* hairs respond only to motion and the electrophysiological response is purely phasic in nature. However, *C. partellus* sensilla respond to any sort of mechanical stimulation and have a typical phasic-tonic response pattern. The central nervous systems of these insects is able to translate these two contrasting physiological response profiles into appropriate behavioral reactions. Yamaoka *et al.* (1971) also found that mechanoreceptors on the anal papillae of *Bombyx mori* are critical to normal egg laying behaviour. Females generally lay a monolayer of eggs in close proximity to one another. However, females with damaged tactile hairs not only scatter their eggs, but also lay in multiple layers. A number of other studies have found that tactile stimulation is important for ovipositing insects (Shorey, 1964; Rice, 1976; Hattori & Sato, 1983; Van Leerdam *et al.*, 1983; Manjulakumari & Geethabali, 1991). Renwick and Radke (1982) suggested that the structure of the leaf may be more important for ovipositing *C. fumiferana* females. Grant and Langevin (1994) also showed that the influence of mechanosensory input overrides close-range chemical cues for spruce budworm. The female budworm's ovipositor has a tremendous number of mechanosensilla. These should provide the females with sufficient details of the physical quality of the host. I propose that integration of both mechanical and chemical sensory input at the level of the CNS allows the female to make the correct choice.

Surface constituents such as epicuticular waxes alone can provide the insect with enough information for selecting an egg laying site (Bernays *et al.*, 1976). Results from the Rivet and Albert (1990) study imply that surface waxes are perceived by the proboscis. Städler (1974) has also shown that volatile stimulants (pinenes) are detected by the antennae of the adult female. Some species are known to drag their ovipositor over the surface of the plant and this behaviour has also been observed in *C. fumiferana* (Albert, unpublished observations). Perhaps the two types of multiporous sensilla on the ovipositor of *C. fumiferana* are more important for host recognition. These multiporous hairs could be sensitive to surface volatiles released during the act sweeping the ovipositor over the leaf surface. Based on the findings

presented, it is concluded that the Type II hairs on the ovipositor do not serve a chemotactic function for detecting surface waxes. Type II sensilla on the ovipositor may be important in other aspects of mating behaviour. These hairs are quite responsive to solutions of male extracts and might also respond to oviposition deterring pheromones from egg washes or larval frass.

# BIBLIOGRAPHY

Albert, P.J. and S. Parisella. 1988. Feeding preferences of eastern spruce budworm larvae in two-choice tests with extracts of mature foliage and with pure amino acids. J. Chem. Ecol. 14 (8): 1649-1656.

Anderson, P. and E. Hallberg. 1990. Structure and distribution of tactile and bimodal taste/tactile sensilla on the ovipositor, tarsi and antennae of the flour moth, *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae). Int. J. Insect Morphol. & Embryol. 19 (1): 13-23.

Angioy, A.M., A. Liscia and P. Pietra. 1983. Cyclic sensitivity variations in the labellar chemosensilla of *Calliphora*. Experientia 39: 546-547.

Baker, G.T. and S. B. Ramaswamy. 1990. Tarsal and ovipositor sensilla of *Heliothis virescens* and *H. subflexa* (Lepidoptera: Noctuidae). Proc. Entomol. Soc. Wash. 90 (3): 521-529.

Bernays, E.A., W.M. Blaney, R.F. Chapman and A.G. Cook. 1976. The ability of *Locusta migratoria* L. to perceive plant surface waxes. *In*: T. Jermy and A. Szentesi (eds), *The Host-Plant in Relation to Insect Behaviour and Reproduction*. Symposia Biologica Hungarica 16: 35-40.

Bowen, M. F. 1991. The sensory physiology of host seeking behaviour in mosquitoes. Ann. Rev. Entomol. 36: 139-158.

Calvert, W.H. and F. E. Hanson. 1983. The role of sensory structures and preoviposition behaviour in oviposition by the patch butterfly, *Chlosyne lacinia*. Entomol. Exp. Appl. 33: 179-187.

Chadha, G.K. and R. E. Roome. 1980. Ovipositon behaviour and the sensilla of the ovipositor of *Chilo partellus* and *Spodoptera littoralis* (Lepidoptera: Noctuidae). J. Zool. Lond. 192: 169-178.

Cuperus, P. L. 1985. Inventory of pores in antennal sensilla of *Yponomeuta* spp. (Lepidoptera: Yponomeutidae) and *Adoxophyes orana* F.v.R. (Lepidoptera: Tortricidae). Int. J. Insect Morphol. & Embryol. 14 (6): 347-359.

Derridj, S, V. Gregoire, J. P. Boutin and V. Fiala. 1989. Plant growth stages in the interspecific oviposition preference of the european corn borer and relations with chemicals present on the leaf surfaces. Entomol. Exp. Appl. 53: 267-276.

Dethier, V. G. 1982. Mechanism of host-plant recognition. Ent. Exp. Appl. 31: 49-56.

Eisemann, C. H. and M. J. Rice. 1985. Oviposition behaviour of *Dacus tryoni*: the effects of some sugars and salts. Entomol. Exp. Appl. 39: 61-71.

Faucheux, M. J. 1988. Multiporous sensilla on the ovipositor of *Monopis crocicapitella* (Lepidoptera: Tineidae). Int. J. Insect Morphol. & Embryol. 17 (6): 473-475.

Faucheux, M. J. 1991. Morphology and distribution of sensilla on the cephalic appendages, tarsi and ovipositor of the European sunflower moth, *Homoeosoma nebulella* Den. & Schiff. (Lepidoptera: Pyralidae). Int. J. Insect Morphol. & Embryol. 20 (6): 291-307.

Feeny, P, E. Städler, I. Ahman and M. Carter. 1989. Effects of plant odor on ovipositon by the black swallowtail butterfly, *Papilio polyxenes* (Lepidoptera: Papilionidae). J. Insect Behav. 2 (6): 803-827.

Grant, G. C. and D. Langevin. 1994. Oviposition responses of four *Choristoneura* (Lepidoptera: Tortricidae) species to chemical and physical stimuli associated with host and nonhost foliage. Environ. Entomol. 23 (2): 447-456.

Grisdale, D.G. 1984. A laboratory method of mass rearing the eastern spruce budworm *Choristoneura fumiferana*. In: King, E.G. and N.C. Leppla (eds), Advances and Challenges in Insect Rearing. U.S. Department of Agriculture Tech. Bull., pp. 223-231.

Hattori, M, and A. Sato. 1983. Substrate factors involved in oviposition response of the limabean pod borer, *Etiella zinckenella* Treitshke (Lepidoptera: Pyralidae). Appl. Ent. Zool. 18 (1): 50-56.

Hedin, P. A. and J. C. McCarty. 1990. Possible roles of cotton bud sugars and terpenoids in oviposition by the boll weevil. J. Chem. Ecol. 16 (3): 757-772.

Hedin, P. A., W. P. Williams, P. M. Buckley and F. M. Davis. 1993. Arrestant responses of southwestern borer larvae to free amino acids: structure-activity relationships. J. Chem. Ecol. 19 (2): 301-311.

Honda, K. 1990. Identification of host-plant chemicals stimulating oviposition by swallowtail butterfly, *Papilio protenor*. J. Chem. Ecol. 16 (2): 325-337.

Honda, K. 1995. Chemical basis of differential oviposition by lepidopterous insects. Arch. Insect Biochem. Physiol. 30: 1-23.

Hooper, R. L., C. W. Pitts and J. A. Westfall. 1972. Sense organs on the ovipositor of the face fly, *Musca autumnalis*. Ann. Entomol. Soc. Am. 65 (3): 577-586.

Judd, G. J. R. and J. H. Borden. 1992. Influence of different habitats and mating on olfactory behaviour of onion flies seeking ovipositional hosts. J. Chem. Ecol. 18 (4): 605-620.

Kapoor, N. N. 1989. Distribution and innervation of sensilla on the mouthparts of the carnivorous stonefly nymph, *Paragnetina media* (Walker) (Plecoptera: Perlidae). Can. J. Zool. 67: 831-838.

Klijnstra J. W. and P. Roessingh. 1986. Perception of the oviposition deterring pheromone by tarsal and abdominal contact chemoreceptors in *Pieris brassicae*. Entomol. Exp. Appl. 40: 71-79.

Kostal, V. and S. Finch. 1994. Influence of backround on host-plant selection and subsequent oviposition by the cabbage root fly (*Delia radicum*). Entomol. Exp. Appl. 70: 153-163.

Landolt, P. J. 1989. Attraction of the cabbage looper to host plants and host plant odor in the laboratory. Entomol. Exp. Appl. 53: 117-124.

Maloney, P. J., P. J. Albert and A. P. Tulloch. 1988. Influence of epicuticular waxes and balsam fir on feeding behaviour of the eastern spruce budworm. J. Insect Behav. 1 (2): 197-208.

Manjulakumari, D. and M.V.V. Geethabali, . 1991. Ovipositonal response of *Bombyx mori* to different textures of the substratum. Entomol. 16 (1): 1-15.

Merritt, D. J. and M. J. Rice. 1984. Innervation of the cercal sensilla on the ovipositor of the Australian sheep blowfly (*Lucilia cuprina*). Physiol. Entomol. 9: 39-47.

Mitchell, B. K. and W. D. Seabrook. 1974. Electrophysiological investigations on tarsal chemoreceptors of the spruce budworm, *Choristoneura fumiferana* (Lepidoptera). J. Insect Physiol. 20: 1209-1218.

Nair, K.S. S. and F. L. McEwen. 1976. Host selection by the cabbage maggot, *Hylemya brassicae* (Diptera: Anthomyiidae): effect of glucosinolates and common nutrients on oviposition. Can. Entomol. 108: 1021-1030.

Nishida, R. & H. Fukami. 1989. Ecological adaptation of an aristolochiaceae-feeding swallowtail butterfly, *Atrophaneura alcinous*, to aristolochic acids. J. Chem. Ecol. 15: 2459-2463.

Ogwaro, K. and E. D. Kokwaro. 1981. Morphological observations on sensory structures on the ovipositor and tarsi of the female and on the head capsule of the larvae of the sorghum shootfly, *Atherigona soccata rondani*. Insect Sci. Appl. 2 (1/2): 25-32.

Ramaswamy, S. B. 1988. Host finding by moths: sensory modalities and behaviours. J. Insect Physiol. 34 (3): 235-249.

Ramaswamy, S. B. 1994. Physiological basis of feeding and oviposition behaviour in moths. *In:* Ananthakrishnan, T. N. (ed), *Functional dynamics of phytophagous insects*. Oxford & IBH Publishing Co. PVT, Ltd, New Delhi, 55-78.

Renwick, J. A. A. and C. D. Radke. 1982. Ovipositional choice and larval survival of the spruce budworm, *Choristoneura fumuferana* (Lepidoptera: Tortricidae). Environ. Entomol. 11: 503-505.

Renwick, J. A. A. and C. D. Radke. 1983. Chemical recognition of host plants for oviposition by the cabbage butterfly, *Pieris rapae* (Lepidoptera: Pieridae). Environ. Entomol. 12 (2): 446-450.

Renwick, J. A. A. and C. D. Radke. 1985. Constituents of host- and non-host plants deterring oviposition by the cabbage butterfly, *Pieris rapae*. Entomol. Exp. Appl. 39: 21-26.

Renwick, J. A. A. and F. S. Chew. 1994. Oviposition behaviour in lepidoptera. Ann. Rev. Entomol. 39: 377-400.

Reymonet, C, and M. J. Faucheux. 1991. Wall-pore sensilla on the ovipositor of the European sunflower moth, *Homoeosoma nebulella* Denis & Schiffermuller (Lepidoptera: Pyralidae), Ann. Soc. Ent. Fr. (N.S.) 27 (1): 119-120.

Rice, M. J. 1976. Contact chemoreceptors on the ovipositor of *Lucilia cuprina* (Weid.), the Australian sheep blowfly. Aust. J. Zool. 24: 353-360.

Rice, M. J. and T. M. McRae. 1976. Contact chemoreceptors on the ovipositor of *Locusta migratoria* L. J. Aust. Entomol. Soc. 15: 364.

Rivet, M.-P. and P. J. Albert. 1990. Oviposition behaviour in spruce budworm *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae). J. Insect Behav. 3 (3): 395-400.

Schnuch, M. and K. Hansen. 1990. Sugar sensitivity of a labellar salt receptor of the blow fly *Protophormia terraenovae*. J. Insect Physiol. 36 (6): 409-417.

Schoonoven, L. M., W. M. Blaney and M. S. J. Simmonds. 1992. Sensory coding of feeding deterrents in phytophagous insects. *In:* Bernays, E., *Insect-Plant Interactions*, Vol IV, CRC Press, Boca Raton, pp. 59-71.

Shorey, H. H. 1964. The biology of *Trichoplusia ni* (Lepidoptera: Noctuidae). III. Response to the oviposition substrate. Ann. Ent. Soc. Am. 57: 165-170.

Smith, J. J. B., B. K. Mitchell, A. T. Whitehead and P. J. Albert. 1990. Sapid Tools: microcomputer programs for analysis of multi-unit nerve recordings. Chem. Senses 15 (3): 253-270.

Städler, E. 1974. Host plant stimuli affecting oviposition behaviour of the eastern spruce budworm. Entomol. Exp. Appl. 17: 176-188.

Städler, E. and W. D. Seabrook. 1975. Chemoreceptors on the proboscis of the female eastern spruce budworm: electrophysiological study. Entomol. Exp. Appl. 18: 153-160.

Städler, E. and H. R. Buser. 1984. Defense chemicals in leaf surface wax synergistically stimulate oviposition by a phytophagous insect. Experientia 40: 1157-1159.

Städler, E. 1986. Oviposition and feeding stimuli in leaf surface waxes. In: Juniper, B. and R. Southwood (eds), Insects and the plant surface, Edward Arnold, London, pp. 105-121.

Stoffolano, J. G. jr. and L. R. S. Yin. 1987. Structure and function of the ovipositor and associated sensilla of the apple maggot, Rhagoletis pomonella (Walsh) (Diptera: Tephritidae). Int. J. Insect Morphol. & Embryol. 16 (1): 41-69.

Tisdale R. A. and M. R. Wagner. 1991. Host stress influences oviposition preference and performance of a pine sawfly. Ecol. Entomol. 16: 371-376.

Tulloch, A. P. 1987. Epicuticular waxes of Abies balsamea and Picea glauca: occurrence of long-chain methyl esters. Phytochem. 26 (4): 1041-1043.

Valencia, L. and M. J. Rice. 1982. Contact chemoreceptors on the ovipositor of the potato moth, Phthorimaea operculella (Zell.) (Lepidoptera: Gelechiidae). Int. J. Insect Morphol. & Embryol. 11 (2): 121-128.

Van Leerdam, M. B., K. J. R. Johnson and J. W. Smith jr. 1984. Effects of substrate physical characteristics and orientation on oviposition by Eoreuma loftini (Lepidoptera: Pyralidae). Environ. Entomol. 13: 800-802.

Waladde, S. M. 1983. Chemoreception of adult stem-borers: tarsal and ovipositor sensilla on Chilo partellus and Eldana saccharina. Insect Sci. Applic. 4 (1/2): 159-165.

Yamaoka, K, M. Hoshino and T. Hirao. 1971. Role of sensory hairs on the anal papillae in oviposition behaviour of Bombyx mori. J. Insect Physiol. 17: 897-911.

Yeh, C. and M. J. Klowden. 1990. Effects of male accessory gland substances on the pre-oviposition behaviour of Aedes aegypti mosquitoes. J. Insect Physiol. 36 (11): 799-803.

Zacharuk, R. Y. 1962. Sense organs of head of larvae of some Elateridae (Coleoptera): their distribution, structure and innervation. J. Morphol. 111: 1-33.

Zacharuk, R. Y., R. M. K. W. Lee and D. E. Berube. 1986. Fine structure of sensilla on the ovipositor of the tephritid fly Urophora affinis. Can. J. Zool. 64: 973-984.

Zhi-Xin, W. and Q. Junde. 1982. Ovipositional response of *Trichogramma dendrolimi* to chemical contents of artificial eggs. Acta Entomol. Sinica 25 (4): 363-372.