RESPONSES OF SOME HOVERFLIES TO OVIPOSITION SITES

by

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ABSTRACT

Antennal sensilla of *Metasyrphus venablesi* (Cn.) and *Eupeodes volucris* O.S. (Diptera: Syrphidae) were studied by scanning and transmission electron microscopy. Males and females both had four types of sensilla. Three of these, two multiporous perforated (MPP) sensilla (one round-tipped and one pointed), and a grooved peg, multiporous sensillum, were also confirmed by SEM on the following species: *Syrphus torvus* (♂, ♀), *Scaeva pyrastrae* (♂, ♀), *Dasysyrphus amalopsis* (♀), *Xanthogramma flavipes* (♀), *Brachyopa perplexa* (♂), *Pipiza sp.* (♀), *Xylota sp.* (♂). The fourth MPP sensillum had thicker walls and fewer pores. All four types were located among dense non-innervated setae on the antennal bulb and appeared to be olfactory.

EAG study of the antennae of female *M. venablesi* and *E. volucris* showed that both species responded to: common green plant volatiles, trans- and cis-2-hexen-1-ol, trans- and cis-3-hexen-1-ol, cis-3-hexenylacetate, and hexanol; other volatile plant substances, methylsalicylate and amylacetate; crushed carnation petals and crushed aphids. There was no response to honeydew or some of its components (e.g. tryptophan, indolealdehyde or indoleacetaldehyde) nor was there a response to water vapour.

A gustatory sensillum on the ovipositor of these two species was studied by scanning and transmission electron microscopy and by neurophysiological methods. One mechanosensitive and four chemosensitive neurons innervate each hair. The chemosensitive neurons are exposed to the exterior by a terminal pore, and respond to honeydew, tryptophan, indoleacetaldehyde, alanine, sucrose, and water. Labellar hairs are also
sensitive to sucrose.

Olfactometer study of *M. venablesi* and *E. volucris* showed that olfactory stimulation by flowers would induce searching by both sexes. A mixture of tryptophan and indoleacetaldehyde induced mated females to search for and locate the stimulus. Other components of the oviposition stimulus—crushed plant, uninfested plant, and aphids did not induce searching. Physiological condition of the insect affected response to aphid-infested plants. Mated females, previously exposed to the stimulus, were more responsive than mated, inexperienced females or unmated, previously exposed females. Unmated, inexperienced females were least responsive. Mated and previously exposed males were more responsive than unmated, inexperienced males.

Elements of the oviposition stimulus were presented on green glass rods to mated females. The attractive elements included fresh dead aphids, honeydew, crushed bean, tryptophan and indoleacetaldehyde, and clusters of black spots similar in size to aphids. Males were attracted only to honeydew and crushed bean. Both sexes responded to potential food sources, such as honeydew, but only females responded to aphids and attractants that characterized oviposition sites. A stimulus-response sequence is proposed for these aphidophagous syrphids that involves dual and/or multiple stimulus combinations.
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GENERAL INTRODUCTION
Insects live in a world that continually requires them to respond appropriately to various types of sensory information. Stimuli that are available to them include light and darkness, light wavelength, sound, the gravitational field, pressure, temperature, odours, tastes and textures. Which of these they respond to depends on their specific requirements for food, shelter, mating, or oviposition.

Some insects are able to use only a limited number of stimuli, whereas others have more complex capabilities, and often utilize combinations of stimuli to initiate or reinforce behaviour patterns. Some stimuli, however, are used almost universally by insects, as evidenced by the widespread occurrence of the special structures required to receive these stimuli. Chemical stimuli (be they volatile or not) belong to this group, and one can find evidence of specialized receptors for chemical recognition on any insect studied.

The antennae are the most obvious location for chemical receptors, and they bear numerous small structures especially adapted for receiving chemical information. These sensilla are not limited to antennae but may also be found on legs, the internal and external mouthparts, and the ovipositor. The wide distribution and diversity of these sensilla in other animals besides insects have led to speculation that chemical recognition was the earliest sensory system evolved (Zacharuk 1980).

Even though insects have such a variety of receptors for distinguishing chemical stimuli, Dethier (1971) pointed out that there is still a paucity of such receptors, considering the rich variety of chemical stimuli available. Insects, like other animals, have evolved chemical sensing devices for detecting stimuli that have adaptive value,
so that most of their receptors are sensitive to a "specific" selection of the stimuli available.

Food aquisition requires first, searching, then recognition, and finally feeding, in response to a sequence of appropriate perceived stimuli. Oviposition also requires perception of sequential stimuli. Egg-laying may be coupled with food, so that the stimulus to feed is also the stimulus to oviposit. Of course, in such insects, adult and larval food supplies must be the same. Having a variety of responses to one set of stimuli is economical both in terms of energy utilization and sensory diversity. Other insects may use different sets of stimuli for each activity, and have equally diverse behavioural responses.

Mating is also commonly linked with food, so that feeding stimuli bring the sexes together. Specific mating stimuli then come into play. Responses to the presence of the opposite sex may be limited to depositing a spermatophore on the substrate near a female (in Collembola, Schaller 1971), or they may be more elaborate, including ritual dancing or gift bearing (e.g. in the scorpionfly Bittacus apicalis , Thornhill 1976) to enhance the female's receptivity.

No matter what degree of complexity may be involved in the initial stimulus or the resulting behaviour, the neural circuitry of insects lack synaptic connections between the sensillum and the brain. Whatever "turns on" a sensillum is transmitted directly to the CNS. This direct linkage makes insects ideal subjects for studying sensory responses at the organismal or the cellular levels.
Searching for an oviposition site involves several sensilla simultaneously or sequentially. The eyes receive visual stimuli, while the antennae, the tarsi, the labellum, and the ovipositor receive chemical information. For most insects, chemoreception is divided into two classes, olfactory and contact chemoreception, although the neural mechanism of recognition is thought to be the same (Zacharuk 1980). Olfaction and contact chemoreception usually take place on different sensilla and often on different parts of the body.

A major prerequisite for interpreting insects' sensory responses to their world is an understanding of the kinds of sensilla which are available to them, and the possible functions these may serve. Chemoreceptors comprise a large proportion of an insect's array of sensilla, displaying great variety in form and function. Insects with different feeding and ovipositional requirements offer a richer behavioural repertoire in response to specific chemical stimuli than those insects which combine these requirements. Insects which have distinctly different behaviours for feeding and oviposition provide better opportunities for testing these behaviours separately. The Syrphidae include excellent examples of this latter type.

The adult syrphids are primarily pollen and nectar feeders (Schneider 1969; Holloway 1976) although some may also utilize aphid honeydew. They search out flowers to provide their own food. Larval syrphids have diverse feeding patterns, but a number are obligate predators of aphids. Since these predatory larvae are blind and move only slowly, the adult females must oviposit in or very near an aphid colony to ensure survival of their offspring. Since the females of these obli-
Gate predators must search for two different food sources, flowers for their own requirements and plants infested with aphids for their offspring. I chose this group for studying the morphological differences among chemoreceptors, and the possible differences in resulting behaviour patterns.

Visually and chemically, sites for feeding and for oviposition may have little in common. Flowers tend to be variously coloured, and many are fragrant. An aphid-infested plant, on the other hand, has a more limited range of colour (i.e. green to yellow) but may retain many of the original chemical characteristics of the plant, as well as signalling the presence of the aphids. The purpose of this thesis is to investigate syrphid responses to these two different sites by:

1. examining the ultrastructure of the chemosensilla of a highly specialized syrphid group;

2. testing behavioural responses of individual insects to various components of each site;

3. further separating identifiable components of any attractive stimuli for additional testing by neurophysiological techniques.
CHAPTER 1. ULTRASTRUCTURE OF CHEMOSENSILLA ON THE SYRPHID ANTENNA AND OVIPOSITOR
Introduction

Chemosensilla are primarily olfactory or gustatory, and these two categories have well defined and quite different structures. The differences are sufficiently marked to allow one to predict the function of a sensillum from its external and internal morphological structure. It was once thought that the olfactory and gustatory categories were mutually exclusive, but Dethier (1972) and Städler & Hanson (1975) have shown that this is not invariably so. In fact, the transition from exclusively gustatory to exclusively olfactory sensilla includes sensilla sensitive to both kinds of stimuli. Morphologically, these transitional sensilla give no indication that they have a dual function. But even when structure may not always denote function, morphological classes are still useful for classifying the majority of sensilla and will be so used here.

Most olfactory receptors of insects are on the antennae, though some have been found on maxillary and labial palps [e.g. in Aedes aegypti (Kellogg 1970) and in the larval beetle, Orthosoma brunneum (White et al. 1974)] and even tarsi [in blackflies (McIver et al. 1980)]. Antennal sensilla have been investigated in many insects, including several of the Diptera: e.g. in mosquitoes (Boo 1980; Boo & McIver 1975, 1976; Jez & McIver 1980; McIver 1972a, 1973, 1974, 1978; McIver & Hudson 1972; McIver & Siemicki 1976, 1978, 1979; Steward & Atwood 1963); blackflies (Elizarov & Chaika 1975; Mercer & McIver 1973); ceratopogonids (Braverman & Hulley 1979; Chu-Wang et al. 1975; Navai & Wirth 1978); tabanids (Elizarov & Chaika 1977); muscid flies (Bay & Pitts 1976; Lewis 1971; White & Bay 1980) and blowflies (Larsen &
Dethier 1963). However, they have not previously been investigated in the Syrphidae.

The following summary gives a generalized description of the structure of olfactory chemoreceptors. Those sensilla responsive to airborne chemicals typically have thin cuticular walls (Slifer 1970) with many pores that allow contact between the dendrite within and the stimulus molecules. These multiporous sensilla (MP after the terminology used by Zacharuk 1980) come in many shapes and sizes: hairs, domes, cones, and pegs, either projecting from the cuticle or surrounded by deep or shallow cuticular pits. The surfaces of these sensilla may have grooves or pits. Pitted-surfaced multiporous (MPP) sensilla are the most common. MPP sensilla are most often found in protected locations (e.g. among other hairs) and in specific distribution patterns on antennae, but also can be found in smaller numbers on other body parts (e.g. ovipositor, palps). These latter receptors may have relatively thick walls with fewer smaller pores, or may have relatively thin walls with larger pores and a higher pore density. The pore may connect with the interior of the sensillum by a straight narrow channel or by flaring into a wider channel - the "pore kettle" of Ernst (1969 in Bay & Pitts 1976). Direct connections with the dendrites may exist in the form of 10-20 nm diameter pore tubules (Zacharuk 1980) extending through the cuticle to the central dendritic chamber.

Within the cuticular covering is a dendritic chamber extending the length of the sensillum. The distal portions of the bipolar neurons extend into this chamber. They may extend as one strand (most common in thick-walled MPP sensilla) or roll, fold, lamellate, or branch in the
thin-walled MPP sensilla. In most insect MPP sensilla, the cuticular dendritic sheath does not reach into the porous part of the sensillum but terminates at its base. There need not be a dendritic sheath in these sensilla (Zacharuk 1980).

The second type of multiporous sensillum has a grooved surface (MPG). These have only been observed scattered sparsely over insect antennae. Most are small and peg-like, with longitudinal grooves extending to the tip of the sensillum. Pores extending into the peg lumen from the grooves may be straight and narrow (giving the grooves an outwardly plain appearance) or have elaborate external orifices that give the grooves an ornate appearance. The dendritic sheath may line the dendritic chamber and be perforated along its length. There are no pore tubules in these sensilla. MPG sensilla usually have 2-5 unbranched sensory neurons (McIver 1974; Chu-Wang et al. 1975).

In both types of MP sensilla, the distal dendritic portion of the neuron extends out from a ciliary region and contains only longitudinal microtubules in a granular cytoplasmic ground substance. From the ciliary region, with its typical microtubule pattern of nine pairs of microtubules surrounding a center without microtubules (9×2 + 0), the dendritic sheath (if it is present) arises and encloses the bundle of dendrites.

The proximal dendritic portion extends below the ciliary and rootlet region to the perikaryon. Within the cytoplasm of this portion of the cell are found inclusions indicative of high metabolic activity, e.g. mitochondria, rough E.R., Golgi bodies, vesicles. The nucleus is characteristically large and round with pale, finely dispersed
chromatin. The axon extends without synapse (Dethier 1971) or fusion (McIver 1978; Moeck 1968; Steinbrecht 1969) to the central nervous system.

There are commonly four (but the range is 2-5) sheath cells of epidermal origin that surround the neuron. Two cells envelope the distal parts of the neuron. The trichogen surrounds the dendrites (and the dendritic sheath if it is present), and perhaps an inner sheath cell, from the perikaryon to near the base of the cuticular portion. The tormogen cell surrounds this cell to the base of the external sensillum. Between them is a sensillar sinus (Zacharuk 1980) or receptor lymph space (McIver 1975). Both sheath cells have cytoplasmic extensions into the sinus. These may be microvilli if showing signs of active secretion, (i.e. mitochondria) or lamellae. The granular fluid in the sinus is believed to be a nutrient source for the dendrites (Gnatzy & Weber 1978; Phillips & Vande Berg 1976) as it bathes them to the external sensory structure. The tormogen cell is believed to sequester nutrients from the haemolymph and secrete them into this sinus.

Interior to the trichogen cell there may be an inner sheath cell enclosing dendrites in a ciliary sinus from the perikaryon to the base of the dendritic sheath. The fluid in this sinus is believed to be secreted by the inner sheath cell and provide nutrients for the dendrites (Bellamy & Zacharuk 1976). The basal sheath cell wraps around the perikaryon and the axon, overlapping the inner sheath cell (if present). If a fifth sheath cell is present, it is wrapped around the tormogen cell and may even secrete into the sensillar sinus.
Gustatory chemosensilla are similar internally to olfactory receptors, but have two notable differences. The two kinds of receptor have in common terminal or subterminal, single or multiple openings through which chemical communication between dendrites and stimulus can occur. But in addition gustatory sensilla often have a mechanosensitive neuron associated with them. These sensilla generally have thick walls and, because of their single opening, are called "uniporous" (UP) sensilla. Those with a simple pore (UPP) are further separated from those with a sculptured pore (UPS).

The pores may contain plugs or exudate which may represent a closure apparatus (Zacharuk 1980). Pore tubules may be present in some UPS sensilla (op.cit). In UPS and UPP sensilla, dendrites usually extend through the lumen of the sensillum encased in a dendritic sheath that is open at the end. In some, there are two channels in the sensillum, separated either by cuticle or by the dendritic sheath.

These gustatory or contact chemoreceptors are most often located on appendages used in contact sensing: e.g. tarsi, labellum, terminal antennal segments, maxillary and labial palps, galea and cerci, and ovispositors. They can have the dual function, as previously noted, of responding to air-borne as well as contact chemicals. Their mechanoreceptive neurons are indistinguishable from chemoreceptive ones, except for their distal tip, which differentiates into an electron-dense "tubular body" of filaments and microtubules before attaching to the wall at the base of the hair (McIver 1975).
Although there is a great deal of literature on the contact chemoreceptors, studies specific to the ovipositor are few, and those concerning Diptera are fewer still (Behan & Ryan 1977; Hooper et al. 1972; Rice 1976). The sensilla of the syrphid ovipositor have never been studied.

This thesis treats the syrphid antenna as the primary organ of olfactory reception, and the ovipositor as an organ of gustatory reception used exclusively for ovipositional stimuli. Their receptors are studied in this chapter by scanning and transmission electron microscopy to elucidate the structures available to certain aphidophagous syrphids for receiving stimuli. These structures can be compared with similar structures in other insects, so that when functional information is available, functions can be suggested for the sensilla described.

Syrphid larvae are also investigated by S.E.M. to predict their sensory sensitivity to aphids, to add to what is already known from one light microscope study (Bhatia 1939) and various behavioural studies (e.g. Chandler 1969; Růžička 1976).
Materials and Methods

Rearing Syrphids

Most species tested were not amenable to laboratory rearing, but Metasyrphus venablesi (Cn.) and Eupeodes volucris O.S. could be reared by Frazer's method (1972) with some minor changes. In addition to the large cages with feeding platforms used by Frazer (op.cit) I also used slightly smaller cages (70 X 60 X 50 cm) for adult flies. Fresh pollen in the form of flowers was supplied when available in addition to freeze dried Corylus sp. pollen. Acyrthosyphon pisum or Aphis fabae were used to induce oviposition. These aphids were reared on broad bean, Vicia faba major cv Broad Windsor, planted in sterile soil. Upon hatching, syrphid larvae were not removed individually from the oviposition plants. Instead, a whole plant was placed in a cage with 9 pots containing 17 cm high broad beans infested with A. pisum so that the larvae could move at random from their original plant. Cannibalism was never a problem unless aphids were scarce. Rearing-room temperature ranged between 22 and 25 degrees C, with a relative humidity of 65%. The cages were illuminated by a combination of warm and cool fluorescent lights with a light/dark regime of 16/8 h.

To avoid further problems with a virus that appeared during the first few months of continuous rearing, all cages were cleaned after each rearing session with bactericidal soap (Bactrex) and 70% ethanol. An independent colony of A. pisum was kept in a cooler room, 10-15 degrees C, with 16 hours light, specifically to infest new broad beans with "clean" aphids. The virus ceased to be a problem with this treat-
ment, so that colonies of *M. venablesi* were kept for 10 to 12 months at a time, and *E. volucris* could be kept for 4 to 5 months. Syrphid generation time under these conditions was 5 to 6 weeks.

Additional species studied here were collected locally before study or reared to the adult stage from eggs. Some were supplied by Dr. J.R. Vockeroth, Biosystematics Research Institute, Ottawa, and these species are noted where results apply to them.

**Scanning Electron Microscopy**

For scanning electron microscopy (SEM) of antennae, either whole heads or patches of cuticle containing antennae were excised from freshly killed syrphids and stored in 70% ethanol (EtOH) until required. Antennae were cleaned by sonication first in a 50% ammonium hydroxide solution for 50 seconds, and then for three consecutive 30 sec periods in pure acetone. The specimens were air dried, mounted on stubs with rubber cement diluted 50/50 with chloroform, and gold coated (Eiko Engineering IB-2 Ion Coater). Other body parts were studied in the same way. A few excised heads were fixed on stubs in 4% osmium tetroxide vapour for 90 min before SEM study, but this lengthy treatment was abandoned, because it produced results very similar to those obtained more rapidly by air drying.

Larvae were preserved in 70% EtOH, dehydrated through an ethanol series to 100% amyl acetate, and critical point dried (Omar SPC 1500 critical point dryer). To obtain larvae with partially extended mouth parts, feeding larvae were killed quickly in 70% EtOH. They were then mounted on stubs with silver-conducting paint and gold coated before
study. All specimens were studied with a Hitachi S 500 Scanning Electron Microscope.

**Transmission Electron Microscopy**

Several methods of fixation and embedding were undertaken in an attempt to improve antennal sections. The most successful is presented here. Specimens were dissected in 5% gluteraldehyde in phosphate buffer (pH 7.0-7.2) on ice, then left overnight in fixative in partial vacuum. Postfixation was in 2% osmium tetroxide (pH 7.0) on ice for one hour. Specimens were slowly dehydrated in ethanol in an ice bath to 70%, then at room temperature from 80% to propylene oxide, all in partial vacuum. Tissue was infiltrated overnight in 50:50 propylene oxide and an embedding medium (Epon 812, Spurr's, Quetol, or Epon-Araldite) in partial vacuum. The following day, specimens were embedded in the appropriate pure resin for polymerization.

Sections of antennae were cut with glass knives, but a few were cut with a diamond knife on a Reichert OM U-2 Ultramicrotome. Ovipositor tissue was treated in the same manner, embedded in Epon 812, and sectioned with a diamond knife.

All sections were stained in uranyl acetate and lead citrate, on collodion, carbon-coated, 100 mesh copper grids. A Philips model 200 or model 300 transmission electron microscope was used to view sections.
Results

Electron Microscopy of Antennae

Syrphid antennae consist of three segments, a scape and pedicel, and a large terminal bulb. The bulb is oval-shaped in cross section and covered with a dense mat of non-innervated cuticular setae. A long slender arista articulates from the laterodorsal margin of the bulb (Plate 1 a). Antennal sensory structures (excluding Johnston's organ) are all located on the antennal bulb.

The most numerous structures on the bulb are the non-innervated setae (Plate 1 b). They are long (mean 13.1 μm ± 0.39, n=4) curved, and spirally grooved, tapering to a fine point. Interspersed among these setae are four types of chemoreceptive sensilla, all multiporous. Because of the dense mat of setae, total numbers of these sensilla could not be accurately counted, but searches for each type soon showed that they varied in density. The two most common were typical multiporous sensilla with exteriors perforated by numerous pores (Plate 1 c). These sensilla differed in size and shape. The more common type had a rounded tip, mean length of 7.78 μm ± 0.31, n=2, and diameter of 1.5 μm ± 0.31, n=9 (Plate 1 c, 2 a). The less common type had a pointed tip, with a mean length about half that of the other type (3.66 μm ± 0.34, n=3) and a diameter at widest point of 1.67 μm ± 0.27, n=5. At the tip, the diameter measured 0.17 μm ± 0.01, n=3. Density of pores was not estimated (Plate 1 c ).
The less common was a grooved peg sensillum of mean length 2.5 μm ± 0.59, n=9, and width at the widest point 0.89 μm ± 0.16, n=11. Each peg had a collared appearance, as it arose from a shallow pit and each had twelve grooves in cross-section (Plate 2b). There were no pores in the distal tip of the peg (Plate 2c). The tips in M. venablesi and E. volucris were blunt (Plate 2c) but in some other species e.g. Xylota sp. (Plate 4b) they have a more pointed extremity.

These three types of sensilla were found in both males and females of M. venablesi, E. volucris, Syrphus torvus and Scaeva pyrastri. There was no appreciable difference in size among sensilla of these species.

The fourth sensillum was also multiporous but was thinner, shorter, and had fewer pores and thicker walls (Plate 2d). These sensilla were only infrequently encountered on the antennal bulb though they occurred in higher density around the laterodorsal margin, in the vicinity of the arista. In this area, the other kinds of MPP sensilla were less frequent. This fourth type of sensillum arises out of a depression in the cuticle, as do the grooved pegs.

Some workers have reported apparent "sensory pits" on the antennal bulbs of syrphids. These pits are much more prominent in some species than in others. In the species examined here, "pits" are visible but not prominent (Plate 1a). Upon examination by SEM, these structures proved not to be "pits", but areas with a thinner population of non-innervated setae, which allowed a clearer view of the underlying sensory structures. Sensilla did not appear more numerous in these "pits".
A specimen of *Brachyopa perplexa* was obtained from Dr. J. R. Vockeroth, Biosystematics Research Institute, Ottawa, for SEM study of its very prominent antennal "pit" (Plate 3 a). Under the scanning electron microscope this round area could be seen to contain a dense population of round-tipped multiporous sensilla (Plate 3 b). Surface cuticle was not visible, as the sensilla were so dense. These sensilla were not confined to the "pit", however, but were also scattered over the entire bulb at a lower density (Plate 3 c). Multiporous round-tipped sensilla and grooved pegs were also found in the following species supplied by Dr. J. R. Vockeroth; a female *Dasysyrphus amalopsis*, a female *Pipiza* sp., a female *Xanthogramma flavipes*, a male *Xylota* sp. and *Brachyopa perplexa* (Plates 3 c, d, 4 a, b).

The two common MPP sensilla, types I and II, could not be distinguished in T.E.M. sections, as crosssectional diameters were very similar and the tip was rarely seen. They will be described together here. Cuticle in the external sensillum was thin (0.15 μm ± 0.04 um, n=10) with numerous pores. Narrowest pore diameter was 32.8 nm ± 6.8 nm, n=10. Pores flared slightly to the exterior and opened into bell-shaped cavities to the interior (Plate 4 d). No pore tubules were present.

Usually three, but sometimes two dendrites, without a dendritic sheath, could be found in the cuticle below these sensilla (Plate 5). As the dendrites entered the base of a sensillum, they branched into many small dendritic extensions (Plate 4 c, Plate 5). The pattern in crosssection was variable (Plate 4 c, d). Often one dendrite remained unbranched or
else rolled upon itself in concentric circles (Plate 4c-d). Dendritic processes were close to but did not appear to enter the flared pore bases (Plate 4d). Sheath cells included a trichogen cell, which wrapped around the distal dendrites until they began to branch (Plate 5). The tormogen cell with its cytoplasmic lamellae lined the outside of the rather limited sensillar sinus (Plate 5). Structures below the cuticle level were not easily distinguishable, due to crowding together of the cellular portions of many sensilla.

Grooved MP sensilla are innervated by three unbranched dendrites (Plate 6c) in a small dendritic chamber. In crosssection, (Plate 6c) the grooves appear as clefts between rounded cuticular flutes. The clefts are rounded at their base and extend the length of the groove. Pores (Plate 6c) are slit-like, extending from the base of the cleft to the dendritic chamber without pore tubules. There is an outer ring of channels with the cuticular flutes giving the sensilla a double-walled appearance (Plate 6a, c).

Below the cuticle, dendrites are enclosed in a thick dendritic sheath (Plate 6a). The trichogen cell does not surround the sheath, except below the cuticle. The tormogen cell with its lamellae lines the sensillar sinus part way through the cuticle but not to the base of the sensillum (Plate 6b). The sensillar sinus is continuous with the dendritic chamber and the cuticle-lined channels. Structures below the thick antennal cuticle could not be delineated because it was difficult to obtain adequate serial sections of antennal material.
The fourth type of MPP sensillum, the thicker-walled sensilla, were rare and consequently only infrequently sectioned. Wall thickness was 0.18 μm ± 0.02 μm, n=10. There were most commonly two dendrites in a sensillum crosssection (Plate 7 c, d) but occasionally 5 were seen (Plate 7 e). From the similar diameters of dendrites, it seems likely that there were two similar sensilla, with different numbers of dendrites rather than dendritic branching.

The pores of these sensilla lacked "pore kettles" (Plate 7 a-d) and had a narrowest diameter of 222 Å ± 52 Å, n=10. The pore channel flared slightly to the exterior and to the interior. Pore tubules could not be seen. In longitudinal and crosssections, there was no dendritic sheath in the large central chamber (Plate 7 a-d). Neither was there a dendritic sheath surrounding distal dendritic extensions in and below the cuticle in most of these sections. There were a few sensilla in this area, however, that did have a dendritic sheath although there were always more than two dendrites inside it. Though evidence is incomplete, this variation supports the suggestion that the 5-dendrite sensillum was not a branched 2-dendrite sensillum but a separate type.
Plate 1. Syrphid Antennal Olfactory Sensilla

A) Scanning electron micrograph of the antenna of a female E. volucris showing faint "sensory pit" areas (sp) and a dense covering of non-innervated setae (nis) on the bulb but not on the scape (s) or pedicel (p). The arista (a) has very few setae.

B) S.E.M. of a male S. opinator antennal bulb showing the grooved non-innervated setae (nis) and thin-walled perforated multiporous (mpp) olfactory sensilla arising out of shallow depressions in the cuticle.

C) S.E.M. of a female M. venablesi antennal bulb showing two types of thin-walled perforated multiporous sensilla; rounded (mpp-1) and pointed (mpp-2).

Legend
a       arista
b       antennal bulb
p       pedicel
sp      sensory pit
nis      non-innervated setae
mpp      multiporous sensillum
mpp-1    multiporous sensillum type 1
mpp-2    multiporous sensillum type 2
Plate 2. Syrphid Antennal Olfactory Sensilla Types

A) S.E.M. of a female *S. opinator* antennal bulb showing surface pores of a multiporous thin-walled sensillum type 1 (mpp-1).

B) S.E.M. of a male *S. pyrastrii* antennal bulb, grooved peg sensillum (thin-walled, multiporous grooved type - mpg).

C) S.E.M. of a female *S. pyrastrii* antennal bulb; grooved peg sensillum tip showing absence of terminal pores.

D) S.E.M. of a female *M. venablesi* antennal bulb with a grooved peg sensillum (mpg) and a thick-walled multiporous chemosensillum (mppt) both arising from cuticular depressions.

Legend

- mpg: multiporous grooved peg sensillum
- mpp-1: multiporous perforated thin-walled sensillum type 1
- mppt: multiporous perforated thick-walled sensillum
Plate 3. Syrphid Antennal Olfactory Sensilla

A) S.E.M. of a whole head preparation of a male B. perplexa showing prominent antennal sensory pits (sp) and extended mouthparts (labellum l).

B) S.E.M. of the antennal bulb of a male B. perplexa sensory pit area showing numerous round tipped multiporous sensilla (mpp-1). Note the absence of non-innervated setae (nis) in the area of the pit.

C) S.E.M. of the antennal bulb of a male B. perplexa some distance from the sensory pit, showing multiporous sensilla (mpp-1), grooved peg sensilla (mpg) and several non-innervated setae (nis).

D) S.E.M. of the antennal bulb of a female Pipiza sp.; multiporous perforated, round tipped sensilla (mpp-1).

Legend

sp  sensory pit
l  labellum
mpp-1  multiporous perforated thin-walled sensillum type
mpg  multiporous grooved peg sensillum
nis  non-innervated setae
Plate 4. Syrphid Antennal Olfactory Sensilla 3

A) S.E.M. of the antennal bulb of a male X. quadrimaculata multiporous grooved peg sensillum (mpg). Note the depression in the cuticle.

B) S.E.M. of the antennal bulb of a female Dasysyrphus amalopsis with round tipped multiporous perforated sensillum (mpp-1) type 1.

s) Transmission electron micrograph of cross sections of several thin-walled multiporous sensilla (mpp type 1 or 2 or both). Note the variable number of dendritic branches (db) and dendrite pattern differences. The sensilla emerging from the cuticle is a mpp and its dendritic branching starts from the point of emergence from the cuticle. A non-innervated seta (nis) is also present.

D) T.E.M. of a cross section of a thin-walled sensilla (mpp) with a large dendrite rolled upon itself, and several other dendritic branches (db). The pores (p) widen internally. Pore tubules are not present but there is a matrix of electron dense material in the sensillar lumen.

Legend

mpg multiporous grooved thin-walled sensillum
mpp-1 multiporous perforated thin-walled sensillum type 1
mpp multiporous perforated thin-walled sensillum
nis non-innervated setae
db dendritic branches
p pore
Plate 5. Longitudinal Section of a Multiporous, Thin-walled Antennal Chemosensillum

T.E.M. of two MPP sensilla arising from the cuticle surface without cuticular collars or depressions. Distal dendrites (dd) are not surrounded by a cuticular sheath and the sensillar sinus (ss) is somewhat limited. The pitted thin-walled cuticle of the external sensillum (pc) can be seen in one sensillum.

Legend

dd  distal dendrites
pd  proximal dendrites
to  tormogen cell lamellae
ti  trichogen cell
pc  pitted cuticle
ss  sensillar sinus
Plate 6. Ultrastructure of a Multiporous Thin-walled, Grooved peg Antennal Chemosensillum

A) T.E.M. of a longitudinal section through a grooved peg sensillum. Dendrites (dd) extend almost to the tip of the peg without branching, and the grooves (g) extend to the tip also. Note the cuticle-lined channels (cc).

B) T.E.M. of a female M. venablesi antennal bulb; longitudinal oblique section through the distal dendrites (dd) of a grooved peg sensillum. There is a dark cuticular dendritic sheath (cs) surrounding the dendrites, and the sensillar sinus (ss) and lamellae of the tormogen cell (to) are visible.

C) T.E.M. of a cross section of a grooved peg sensillum. Note the three unbranched dendrites (dd), cuticle lined spaces (cc), and the pore between the the grooves (p).

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<td>P</td>
<td>pore</td>
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Plate 7. Ultrastructure of the Thick-walled Multiporous Antennal Olfactory Sensillum

A) T.E.M. of a female *E. volucris* antennal bulb, longitudinal section of a thick-walled multiporous sensillum. Note the thick walls (tc) with straight pores (p) without pore kettles, and dendrites (dd) in the sensillar lumen.

B) T.E.M. of a female *E. volucris* thick-walled multiporous sensillum, longitudinal section showing irregular undulations of dendrites (dd) and pores (p) only slightly flared to the interior of the sensillum.

C) T.E.M. cross section of a thick-walled multiporous sensillum with two dendrites (dd) and a single long pore (p).

D) T.E.M. cross section of a thick-walled multiporous sensillum with two dendrites (dd) and several pores (p).

E) T.E.M. cross section of a thick-walled multiporous sensillum with 5 dendrites (dd) and several pores (p). Note the absence of a dendritic sheath.

Legend

tc  thick cuticular walls  
p  pore  
/dd  distal dendrites
Electron Microscopy of Ovipositor Sensilla

The syrphid ovipositor is telescopic, with two pads that normally cover the gonopore (Plate 8 a). These pads have four types of setae and hair. There are very long, pointed straight hairs around the interior margins of the pads (mean length 111.2 \( \mu m \pm 12.8 \mu m \), \( n=11 \); Plate 8 b). Numerous medium-length, slender, curved, contact chemosensilla with slightly spatulate tips (Plate 8 b, 9 a) are dispersed over most of the surface of the pads. A third type, and least frequent of the four kinds of setae found on the pads, is a short, finely-pointed hair or peg, in a pit (Plates 8 c, d). The fourth type forms a dense mat of pointed, curved, non-innervated microtrichia-like hairs (Plates 8 b, c). The medium-length curved hair has a pore or pores at its distal extremity (Plate 9 b, c) and has a mean length of 58.3 \( \mu m \pm 8.9 \mu m \), (\( n=15 \)). These medium-length hairs responded as contact chemoreceptors in electrophysiological tests (Chapter 3) and thus were the sensilla selected for sectioning and further study here.

These sensilla have a typical thick-walled uniporous structure (the "thick walled hairs" of Slifer 1970). The mean pore size is 0.55 \( \mu m \pm 0.12 \mu m \), \( n=6 \). Exterior hair walls are smooth at the base, becoming narrower and shallowly grooved as they taper to a spatulated end. There are 12 grooves. One mechanosensitive and four chemosensitive dendrites innervate the sensillum. The mechanosensitive dendrite differentiates into a tubular body (Plate 10) and, with its dendritic sheath, separates from the others, attaching to the cuticle at the level of the distal edge of the inner collar (Plate 10). The remaining five dendrites do not branch, but pass to the tip of the hair in the small dendritic cav-
ity of the sensillum (Plate 11). The larger cavity is empty, save for fluid continuous with that in the sensillar sinus.

Distal dendrites contain tubules of 31 nm ± 4.9 nm, n=21, diameter. There are cellular components in the proximal dendrite segments; microtubule fragments, ribosomes and mitochondria. The ciliary body has a typical 9x2 + 0 configuration of microtubules (Plate 12b). Branches of the dendritic sheath arise in the ciliary region and fuse to form a continuous dense sheath of 0.1 μm ± 0.04 μm, n=10, thickness. The sheath has invaginations that sometimes separate dendrites in the cluster (Plate 13a) but these invaginations do not continue up into the hair. The tubular body, however, is separated in its own sheath soon after differentiation (Plates 10, 13b). The sheath fuses with the cuticle at the base of the hair - its electron-dense dark "colour" separating it as though into branches again- and eventually it fuses with the sensiller cuticle. Examination of the inner wall of the dendrite chamber shows that the dendritic sheath lines the lumen to the tip (Plate 11).

An inner sheath cell surrounds the dendrites up to the dendritic sheath level, supplying a thin granular matrix medium easily visible when the dendritic sheath becomes continuous (Plate 10). Below the ciliary region of the dendrites, this cell becomes invaginated and displays microvilli- like protrusions into the fluid-filled cavity (Plate 12a).
The intermediate sheath cell (or trichogen) lines the internal cavity of the sensillar sinus, wrapping itself around the dendritic sheath. Numerous long, slender lamellae characterize the walls of this cell (Plate 10). The outer sheath, or tormogen cell, makes up the outer lining of the sensillar sinus. Differences between the liquid in this sinus and that surrounding the dendrites are slight, if present at all. The sensillar sinus liquid appears to have coarser granules. Septate desmosomes also occur in the proximal dendrite walls (Plate 12a). Neurons have large, round nuclei and can be distinguished from sheath cells by their denser cytoplasm, which contains more abundant rough endoplasmic reticulum (Plate 10). The large neuronal nuclei were situated approximately 15.1 μ ± 2.2 μ (n=3) below the base of the collar.
Plate 8. Syrphid Ovipositor Sensilla

A) S.E.M. of a whole ovipositor of M. venablesi. The gonopore (gp) is covered dorsally by two ovipositor pads (op).

B) S.E.M. of ovipositor pads of M. venablesi with four types of hair; long setae (ls), medium length slightly recurved uniporous chemoreceptors (up), pointed hairs in pits (hp), and non-innervated short setae.

C) S.E.M. of the ovipositor pad of M. venablesi. Note cuticular collar (cc) around UP sensilla, the mat of short non-innervated setae (ss) and the prominent depressions surrounding the pointed hairs in pits (hp).

D) S.E.M. of the ovipositor pad of M. venablesi showing a pointed hair in its pit (hp). Note its wide base in contrast to the narrow pointed tip.

Legend

go gonopore
op ovipositor pad
ls long setae
ss short setae
hp hairs in pits
up uniporous chemoreceptors
cc cuticular collar
Plate 9. Uniporous Ovipositor Contact Chemosensillum

A) S.E.M. of *M. venablesi* ovipositor contact chemoreceptor. Note the curved tip and shallow grooves.

B) S.E.M. of *M. venablesi* ovipositor uniporous contact chemoreceptor. The pore (p) on the terminal tip is apparent.

C) S.E.M. of *M. venablesi* ovipositor up contact chemoreceptor with three pores (p) on its distal tip.

Legend

p  pores
Plate 10. Ultrastructure of the Uniporous Ovipositor Chemosensillum

Longitudinal T.E.M. through a contact chemosensillum on the ovipositor pad of M. venablesi. Distal dendrites (dd) are enclosed by a cuticular dendritic sheath (cs) and the mechanoreceptor tubular body (tb) is enclosed in its own sheath. There is a cuticular collar (cc) surrounding the sensillum. Note the variation in cuticular structure allowing for movement of the hair.

The sensillar sinus (ss) is large and lined on the inside by the trichogen cell with its lamellae (tr) and on the outside by the tormogen cell and its lamellae (to). Tormogen (ton) and trichogen (trn) nuclei are distinguishable from the neuron nuclei (nn) in having lighter coloured contents. Likewise, their cytoplasm is less dense than that of the neurons.

Legend

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Plate 11. Ultrastructure of the Uniporous Ovipositor Chemosensillum 2

T.E.M. cross section of a uniporous contact chemosensillum on the ovipositor pad of M. venablesi. Five unbranched dendrites (dd) fill the dendritic chamber (dc). The large crescent-shaped chamber (lc) is continuous with the sensillar sinus below the cuticle. A fine dendritic sheath layer (cs) lines the dendritic chamber.

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<td>lc</td>
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Plate 12. Ultrastructure of the Uniporous Ovipositor Chemosensillum 3

A) T.E.M. cross section through the proximal dendrites (pd) in a UP contact chemosensillum on the ovipositor pad of M. venablesi. The five dendrites are surrounded by a highly invaginated inner sheath cell (is). Desmosomes (de) are numerous between dendrites and the inner sheath cell.

B) T.E.M. cross section through the ciliary region of the dendrites of the UP contact chemosensillum on the ovipositor pad of M. venablesi. Microtubules (t) are organized into the typical 9X2+0 arrangement.

Legend

t  neurotubules
pd  proximal dendrites
is  inner sheath cell
de  desmosome connections
Plate 13. Ultrastructure of the Uniporous Ovipositor Chemosensillum 4

A) T.E.M. cross section through undifferentiated distal dendrites (dd) of a UP contact chemosensillum on the ovipositor of M. venablesi. The cuticular dendritic sheath (cs) is invaginated around individual dendrites. The trichogen cell (tr) surrounds the cuticular dendritic sheath and forms the inner lining of the sensillar sinus (ss). The outer lining is formed by the tormogen cell (to). Exterior to the tormogen cell is a thick layer of material containing microtubules and desmosomes. The section is slightly oblique, cutting through cuticle (c) on only one side.

B) T.E.M. cross section through differentiated distal dendrites (dd) just below the base of the UP contact chemosensillum on the ovipositor pads of M. venablesi. The tubular body (tb) has differentiated completely and is separated into its own cuticular sheath. It is fused to the cuticle on one side of the cuticular wall (c) distal to this section but it has almost made contact. Both trichogen (tr) and tormogen (to) cells are nearing the distal limit of their extension.

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Scanning Electron Microscopy of Larvae

Second- and third-instar larvae of *M. venablesi* were used to study body sensory structures. *M. venablesi* and *S. opinatar* larvae were used to study sensilla associated with the mouthparts.

Larvae are covered with a very thin wrinkled cuticle which has many smooth, roundish, tubercular warts (Plate 14c) and rows of non-innervated cuticular setae arranged in rows (Plate 14b). The cuticle, when stretched smooth by larval movements, shows only the largest, most prominent hairs (Plate 14a). These hairs have a cup-shaped depression or pit at their base (Plate 14d), though no external sensory structure was associated with the pit. These large hairs were found in a crown at regular intervals surrounding the pseudocephalon and thoracic regions. The shorter setae, but not the cupped hairs, were found on the ventral surface, from the first pair of pseudopods to the posterior tip of the larva. There are what appear to be mucous gland openings on the ventral surface just lateral to each of the six pairs of pseudopods on abdominal segments one through six (Plates 15a, b).

Mouthparts are normally retracted into the buccal cavity. The pseudocephalon has its anterior portion divided into two lateral lobes, one on each side of a median groove (Plates 15c). The two smaller lobes at the apex of each cephalic lobe are the larval antennal lobe (lying next to the groove) and the more peripheral maxillary palp lobe (Plate 15d). The antennal primordium possesses two structures; a slender, hair-like protruberance and a peg-like knob, both arising from pits (Plate 16a). The maxillary palp is also rounded, but covered with numerous papillae and some peg-like structures, again in pits (Plate 16.
b). Each papilla has its own toroid or "doughnut-shaped" collar (Plate 16 c).

One other structure of probable sensory function was located near the mouth, at the base of the pseudocephalic protrusion (Plate 16 d). This is probably the sensory papilla described by Bhatia (1939). This papilla was unlike the maxillary papillae, being larger (approximately 10 μm in diameter) with a conspicuous pore at its apex. Because its visibility depended on good extension of the thoracic segments, this papilla was seen only once.
A) S.E.M. of a second instar M. venablesi larva, anterior dorsal surface. Prominent cupped hairs (ch) are arranged in rows circumferentially around the larva. Cuticular spines (cs) are numerous between rows of cupped hairs.

B) S.E.M. of a second instar S. opinator larva with anterior extended. Anterior cuticle is stretched smooth showing prominent long cupped hairs (ch). Pseudocephalic lobes (pl) are seen extended from the buccal cavity.

C) S.E.M. of a second instar M. venablesi larva, dorsal cuticle. Note the tubercular warts (tw) on the cuticle.

D) S.E.M. of a third instar M. venablesi larva with long cupped hairs (ch) standing amid numerous smaller cuticular spines (cs).

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Plate 15. Syrphid Larva 2

A) S.E.M. of the ventral surface of a second instar M. venablesi larva with pairs of pseudopods (pp) and probable lateral mucous glands (mg).

B) S.E.M. of a probable ventral mucous gland (mg) opening on the ventral surface of a second instar M. venablesi larva.

C) Anterior S.E.M. view of S. opinator larva head showing oral opening (o) and two pseudocephalic lobes (pl).

D) S.E.M. of pseudocephalic lobe of S. opinator larva showing detail of the maxillary lobe (ml) and the antennal lobe (al).

Legend

pp pseudopods
mg mucous glands
o oral opening
pl pseudocephalic lobe
ml maxillary lobe
al antennal lobe
A) S.E.M. of the antennal lobe on the pseudocephalic lobe of a larva of *S. opinator*. Note two sensory structures; the peg (pg) and hair (h) both arising from pits.

B) S.E.M. of the maxillary lobe on the pseudocephalic protrusion of the larva of *S. opinator*. Note two types of sensory structure; a peg in a pit (pg), and papillae (p).

C) S.E.M. of the maxillary lobe papillae on the pseudocephalic lobe of a larva of *S. opinator*. Note the "doughnut" collar structure around each papilla (p).

D) S.E.M. of a papilla of probable sensory function (sp) near the base of the pseudocephalic protrusions of the larva of *S. opinator*. Note the prominent central pore.

Legend

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>al</td>
<td>antennal lobe</td>
</tr>
<tr>
<td>pg</td>
<td>peg in a pit</td>
</tr>
<tr>
<td>h</td>
<td>hair in a pit</td>
</tr>
<tr>
<td>ml</td>
<td>maxillary lobe</td>
</tr>
<tr>
<td>p</td>
<td>papillae</td>
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<tr>
<td>sp</td>
<td>sensory papilla</td>
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Discussion

Antennal Sensilla

Antennal sensilla of the syrphids studied here contained no apparent mechanoreceptors or contact chemoreceptors. All receptors studied appeared to be olfactory in nature. This specialization has been reported for other higher Diptera (Slifer & Sekhon 1964; White & Bay 1980).

The antennae of the Brachyceran Diptera differ strikingly from those of Nematoceran forms (e.g. mosquitoes). Species of higher Diptera studied for antennal olfactory structures include the calliphorids Phormia regina (Dethier et al. 1963) and Calliphora vicina (Kaib 1974), and the sarcophagid, Sarcophaga argyrostoma (Slifer & Sekhon 1964).

The absence of chemosensory sensilla on the dipteran arista has been noted by others (Bay & Pitts 1976; Dethier et al. 1963; Lewis 1971; White & Bay 1980). No structure of a chemosensitive nature has been reported on the scape or pedicel. Setiferous plaques present on at least 29 species of muscoid Diptera have been eliminated as olfactory receptors (Greenberg & Ash 1972). Other studies have assumed chemoreceptors are lacking from these two basal subsegments (Bay & Pitts 1976; White & Bay 1980). In the present S.E.M. study of syrphids, no superficial structure with apparent chemosensory capabilities was found on the arista, pedicel or scape.

1. Pitted-Surfaced, Multiporous Sensilla
Thin-walled, pitted multiporous sensilla of types I and II (MPP or sensilla basiconica) found in this study, bear a resemblance to those commonly found in other Diptera (Bay & Pitts 1976; Dethier et al. 1963; Lewis 1971; Slifer & Sekhon 1964; White & Bay 1980) in size, wall thickness, number of neurons and dendritic branching. Their pore structure, however, is unusual. Whereas other flies studied all had pore kettles and pore tubules associated with each cuticular perforation, pores of the present sensilla widen into a bell-shaped cavity continuous with the lumen of the sensillum. Consequently there is no pore tubule to complete the connection through cuticle. This arrangement of pores however, was reported for sensilla trichodea of the sand fly, Culicoides furans (Chu-Wang et al. 1975). The absence of a dendritic sheath has been observed in the previously mentioned sensilla of C. furans (op.cit) and also in the face fly, Musca autumnalis (Bay & Pitts 1976).

The variation in pattern of dendritic branching within the sensillum was also observed by Lewis (1971) in Stomoxys calcitrans. According to Lewis, a finely branching dendrite in an olfactory sensillum is likely to be more sensitive than a larger unbranched one. This difference therefore may indicate variable sensitivity in the receptor cells. The functional significance of concentrically layered dendrites has not been elucidated, but it has been suggested that the increased surface area similarly increases the receptive surface (McIver 1972). Various lamellated and "rolled" arrangements of dendrites have been reported in thin-walled pegs (sensilla basiconica) on the palps of female culicine mosquitoes (McIver 1972b), in pegs in pits (sensilla coeloconica) on the antennae of culicine mosquitoes (McIver 1973), in the bulb-shaped sensilla of the palps of C. furans (Chu-Wang et al. 1975) as well as in the
apparently olfactory clavate sensilla (with pitted surface) on the antenna of *S. calcitrans* (Lewis 1971). Only in the sandfly and culicine mosquitoes are sensilla with lamellate dendrites known to function as carbon dioxide receptors (Chu-Wang *et al.* 1975; McIver 1973).

2. Grooved-Surface, Multiporous Sensillum

The grooved pegs in this study were surface structures, never found in pits, as they have been for *C. furans* (Chu-Wang *et al.* 1975) and *Anopheles stephensi* (Boo & McIver 1976). This surface arrangement is also common among other flies studied (Bay & Pitts 1976; Dethier *et al.* 1963; Lewis 1971; Slifer & Sekhon 1964; White & Bay 1980). The number of grooves found here, 10-12, is small compared with the ranges reported for other Diptera, 10-16. Pores are a common feature in these sensilla, and only in one species, *Aedes aegypti* (McIver 1974) do terminal pores replace pores along the grooves. Groove-pores have no pore tubules (Zacharuk 1980) but are considered olfactory receptors because of neurophysiological evidence (Kellog 1970 for those with terminal pores, and Altner *et al.* 1973, in Zacharuk 1980, for multiporous types). The number of unbranched dendrites, 3, found in the present study is within the range of 1 to 5 found in other dipterans studied. In only one dipteran have dendrites been found to branch in these sensilla (in *A. stephensi*, Boo & McIver 1976). MPG sensilla found here were also shorter than most by about half, giving them a short stout appearance. As in comparable sensilla previously studied, the dendritic sheath in these MPG sensillum extended all or part of the way up into the peg lumen.
Grooved-surfaced MP sensilla are a common feature on dipteran antennae, whether these are bulbous, as in the syrphid antenna, or flagellar, as in antennae of mosquitoes and blackflies (Boo & McIver 1976; McIver 1974; Mercer & McIver 1973). The functions of grooved pegs have been studied neurophysiologically in mosquitoes. Kellogg (1970) found grooved pegs of *A. aegypti* responded to ammonia, anisole, to water vapour. This latter observation, however, could not be confirmed for either males or females by Davis (1977) or Davis & Sokolove (1976). In females, the MPG sensillum has responded to lactic acid (Davis & Sokolove 1976), fatty acids and essential oils (Lacher 1967) and commercial repellents (Davis & Rebert 1972). In males, this same sensillum did not respond to the repellents (Davis 1977). Morphologically similar types of sensilla, therefore, do not necessarily function similarly, a phenomenon that has also been noted in the tobacco horn worm, *Manduca sexta* (Stadler & Hanson 1975). In this caterpillar, a group of apparent contact chemoreceptors on the maxillae responded to olfactory odours of their food plant, whereas a morphologically identical second group gave no response until they came into contact with the food source. Stadler & Hanson (1975) concluded that receptor sensitivity varied among sensilla, which may explain the sex differences in responses to repellents by male and female *A. aegypti*.

3. Thick-Walled, Multiporous Sensilla

The thickness of the cuticular walls of these sensilla is actually not much greater than those of the other multiporous sensilla on the antennae (0.18 μm as opposed to 0.15 μm). The difference arises from the form of the pores. Because of the area taken up by the bell-shaped
inner pore (see Plate 4 d) in the MPP types I and II sensilla, many parts of the cuticle are much thinner than the reported mean thickness. In contrast, the pores were fewer and much narrower throughout their length in the "thick-walled" sensilla, so that the thickness of most parts of the intervening cuticle is close to the mean value (see Plate 7 d, e). In fact, our "thick-walled" MPP sensilla fall just short of the range given by Zacharuk (1980) for wall thickness of this type of sensillum (0.2-1.0 μm).

Although this MPP sensillum was not tapered to a point, it appears from the literature that, in other ways, it is most similar to the sensilla trichodea of other authors. These sensilla trichodea tend to be "hairs", sometimes pointed, sometimes blunt, and often curved. Uniporous sensilla are sometimes included in this category but only those MPP sensilla reported for dipterans are considered in this discussion. Lengths of these sensilla vary, from 11 μm (A. aegypti, McIver 1978) to 83 μm (Phormia regina, Dethier et al. 1963) and often one species has two or more lengths (Lewis 1971; McIver 1978; McIver & Siemicki 1979).

Dendrites in this sensillum were unbranched. They have been reported branched (Bay & Pitts 1976; McIver 1978; McIver & Siemicki 1979) and unbranched (Dethier et al. 1963; Lewis 1971; White & Bay 1980) in comparable sensilla. In the present study the pore channel was the same diameter throughout (approx. 222 Å) but this is usually not the case in other thick-walled MPP sensilla. White & Bay (1980) reported a "V" shaped channel widening to the interior in Haematobium irritans irritans, as did Bay & Pitts (1976) for M. autumnalis. The absence of pore tubules noted in syrphids was also recorded in A. aegypti (McIver...
1978; McIver & Siemicki 1979) but pores are present in H. i. irritans (White & Bay 1980) and M. autumnalis (Bay & Pitts 1976). Even density of pores varies considerably in the species in which it has been reported (2-20 per square micron, Zacharuk 1980). Clearly, this type of sensillum, for which there is least information, is also the most variable, according to the literature. Because of the porous nature of their cuticle, these sensilla are classed as olfactory (Zacharuk 1980) but I cannot speculate further on function, because of the variability recorded in the literature.

Uniporous Ovipositor Sensillum

The uniporous sensillum described from the syrphid ovipositor is a typically gustatory or contact chemosensillum of the thick-walled type of Slifer (1970) and the UPP (uniporous with simple pit pore) chemosensilla type of Zacharuk (1980). In some UPP sensilla, the dendritic sheath encloses the dendrites in the dendritic channel (e.g. Felt & Vande Berg 1976). In others (e.g. Cook 1972) as well as these syrphids, it is fused with the inner surface of the dendritic canal (see Plate 11) and there is no separate sensillar channel.

The ovipositor of the facefly, M. autumnalis, has been studied for contact chemoreceptors (Hooper et al. 1972). One receptor very similar to the one described herein was present. It was shorter, 35 μm, than that on M. venablesi, 58.3 μm, but contained 5 neurons, one being mechanoreceptive. The other four continued unbranched to the terminal pore. There was a second sensillum with 3 dendrites, all enclosed in their own dendritic sheaths to the terminal pore. Other Diptera that
have shown evidence of contact chemoreceptors on their ovipositors are: the blow fly, *Lucilia cuprina* (Rice 1976); the carrot fly, *Psila rosae*; and the cabbage root fly, *Delia brassicae* (Behan & Ryan 1977).

**Larval Sensilla**

Syrphid larvae appear to have gustatory receptors on their maxillary lobe as well as in the buccal cavity when viewed with the scanning electron microscope. This arrangement confirms Bhatia's (1939) report based on light microscopy. It also supports the behavioural observation that *S. corollae* larvae have definite feeding preferences (Růžička 1976). When given a choice of different aphids, these larvae consistently chose the same prey. They avoided *Cavariella theoboldi*, which is toxic to them (op.cit). Růžička (1976) concluded that larvae chose their food on the basis of aphid "morphological protection" and "nutritional suitability", or taste. The larval antennal lobes seen in the present study also possessed structures of possible sensory capacity, although behavioural evidence of olfactory abilities in larvae is lacking. It is possible that these antennal sensilla also are used in assessing the suitability of prey at short ranges (e.g. a few mm).
CHAPTER 2. OVIPOSITIONAL BEHAVIOUR OF APHIDOPHAGOUS SYRPHIDS
Introduction

Much study has been devoted to the oviposition behaviour of adult syrphids because of their potential for helping to control aphid populations (Schneider 1969). Field studies have shown that ovipositing females have height preferences (Chandler 1968d), and that there are predictable numerical relationships between the size of an aphid colony and the number of eggs laid in or near it (Chandler 1968b; Dixon 1959; Yakhontov 1966). Syrphid females are not deterred from ovipositing in a colony by the presence of other eggs or larval defecation (Chandler 1968c). Although the presence of flowers in an area encourages females to search there for oviposition sites (Schneider 1969), Chandler (1968c) found that flowers in the immediate vicinity of a plant did not influence the oviposition preference of syrphids in large outdoor cages. Some syrphids oviposit preferentially on certain aphid species, but this has more to do with habitat requirements than with prey specificity (Dusek & Laska 1966).

In laboratory studies, the site preferences of ovipositing syrphids have been studied by a number of investigators. The spatial location and exposure of an aphid colony, e.g. on vertical surfaces, are important (Dusek & Laska 1966; Sanders 1980). Syrphid females are attracted to an optical pattern resembling aphids painted on host plants (Chandler 1968b). *Syrphus* spp. were very particular about the size of aphid patch in which they would oviposit, avoiding densities both too low and too high (op. cit).
Host plant effects have, at times, seemed unimportant (Bombosch & Volk 1966; Peschken 1965) but the controversy seems to have been resolved by Chandler (1966, 1968a). When he worked with several syrphid species, he found that one group required only the host plant for oviposition, whereas aphids were the most important stimulus for another group. Female age and previous "aphid abstinence" are also factors (Schneider 1969). It is possible that closer examination of the species, their ages, and the length of time they have been deprived of oviposition stimuli may explain these apparent differences in results.

Colour, as it relates to the oviposition site, has sometimes been shown to be unimportant (Bombosch & Volk 1966; Peschken 1965) but has also directly affected the number of eggs laid on aphid-smeared glass rods (Dixon 1959). Chandler (1968a) considered it important. Pesken (1965) concluded that the most important guiding principle in the orientation of gravid female *S. corollae* was negative phototaxis. More recently, Sanders (1980), confirmed that *S. corollae* preferred to oviposit in shaded colonies when given a choice between colonies in light and shade.

Aphids and their "exudates" (honeydew and alarm pheromone) have perhaps the greatest importance in ovipositional attraction. Volk (1964 in Schneider 1969) isolated, but did not characterize, a single chemical from "aphids and their exudates" that could induce syrphids to oviposit. Honeydew alone was also shown to be a sufficient stimulus for *S. corollae* to oviposit on an artificial substrate (Bombosch & Volk 1966). When honeydew was the oviposition stimulus, Bombosch and Volk (1966) concluded that the position of the egg was determined by the female's body
position and the texture of the substrate as detected by the ovipositor. When aphids themselves were the stimulus, the ovipositor was used to select the site, regardless of substrate texture. The ovipositor was also implicated as a stereotactic organ in Chandler's (1968a) study. Dixon (1959) was able to induce *S. luniger* to oviposit on green, white, and black glass rods smeared with aphids. These syrphids also laid eggs on opaque green vials containing aphids, apparently in response to odour alone. The aphids in the vials were equally effective dead or alive, thus eliminating possible auditory cues from live aphids.

Adult green lacewings (Chrysopidae) searching for aphids are faced with the same problems confronting female syrphids. Honeydew has been shown to be an important factor for chrysopids also, perhaps even more so than for syrphids, since honeydew and pollen constitute adult food (Hagen et al. 1976). Hagen et al. (op. cit) field-tested ten constituent amino acids of honeydew and found tryptophan to be the attractive source (or kairomone) for *Chrysopa carnea*. Because of the low volatility of tryptophan, van Emden & Hagen (1976) also tested a number of oxidation products of tryptophan in an olfactometer. Female *C. carnea* responded maximally to indoleacetaldehyde whereas they were not attracted to the precursor of indoleacetaldehyde, indolealdehyde. Since indoleacetaldehyde is more volatile than tryptophan, van Emden & Hagen (op. cit) postulated that the slow oxidation of tryptophan under field conditions would supply a constant source of the attractive component, indoleacetaldehyde, for searching lacewings. The common factor, honeydew, in the array of substances attracting both syrphids and chrysopids suggests that tryptophan oxidation products should be tested as possible chemical attractants for syrphids.
There are some apparent discrepancies in the literature concerning important visual and chemosensory responses in syrphids. In this chapter, I intend to investigate this problem, first with olfactometer experiments designed to eliminate visual and gustatory cues. Secondly, following Dixon's (1959) example, I shall observe syrphid responses to glass rods pretreated with various components of the total aphid-infested-plant oviposition complex. The experiments with rods will complement the olfactometer studies by offering various combinations of olfactory, gustatory and visual cues, which are still not so complex a stimulus as an aphid-infested plant. The responses of mated and unmated males and females will be observed to determine whether the responses are innate, or related to the insects' physiological (i.e. gravid) state.
Materials and Methods

Olfactometer Experiments

A modified version of Osgood & Kempster's (1971) olfactometer was used in these experiments (Plate 17). In the present model, air was filtered through charcoal before entering the test chambers (or a fresh supply was used while air from within the system was evacuated from the room). All joints in the clear plastic were sealed with masking tape to maintain the desired air flow. White paper was placed in front of the lower part of the test chambers to eliminate any visual cues. Coloured, translucent (or transparent) paper was fixed over the air mixing zone between the test chambers and flight area. The air speed was maintained at a constant flow rate for all tests with a squirrel fan and rheostat set at 30 units.
This figure shows the plexiglass olfactometer with its large flying chamber (fc) for introduction of insects, the air mixing zone (am) over which was placed coloured paper, mesh screens (cm), and two stimulus chambers (sc), with its air circulation system; charcoal filter (cf) and fan (sf).

Legend

fc  large flying chamber
am  air mixing zone
sc  stimulus chamber
cm  copper mesh screen
cf  charcoal air filter
sf  squirrel fan and rheostat
The olfactometer was placed under two warm and two cool fluorescent lights with their long axes oriented to the long axis of the olfactometer. From one to ten insects were introduced for one trial. Preliminary experiments showed that two hours was sufficient for the insects to respond to a stimulus. This period was adopted as the minimum for each experiment. Only first- and second-generation laboratory-reared insects were used. Individuals were never used more than twice and none was ever tested twice in a 24-hour period. Only a few individuals were tested twice with the same stimulus. Mated females, no matter what their response, were also tested for their "egg-laying response" after removal from the olfactometer. They were placed individually in vials containing aphids on a leaf and were left for 4 hours (or overnight if the trial concluded late in the day) after which any eggs laid were counted. Unmated females and males were returned directly to their maintenance cages after an experiment. Age, generation, mating status, time of day, duration of experiment, type of stimulus, and type of paper used over the mixing zone were recorded.

Most experiments were conducted in a rearing room that had the ranges of temperature and humidity already noted (Chap.1 Materials and Methods). A few experiments were conducted in a smaller, environmentally controlled room with comparable conditions of light, temperature and humidity.

The Z test was used here as a common statistic for binomial experiments (eg. response vs no response) and is based on the normal approximation. The statistic was calculated; Proportion observed (Po) - Proportion expected (Pe) divided by standard error of the parameter. If Z
is large, the difference $P_0 - P_e$ must be significantly greater than zero and if $Z$ is small, the difference is less than zero. The hypothesis tested is then, $H_0 : P_0 = P_e$ and $H_1 : P_0 = P_e$. Tables of $Z$ values are used to determine if $H_0$ is rejected, and $P < 0.01$ is the probability that the observed result occurred by chance. In these experiments, $P_e$ was calculated from the overall response to control chambers in all experiments.

**Coloured Glass Rod Experiments**

Nine mm diameter glass tubing was cut into 40 cm lengths and coloured paper (also used for coloured flowers—see Appendix 1) was cut, rolled and inserted into the tube. The tubing was fitted into a 2.5 cm high black rubber cork or a block of plasticine covered with parafilm. The resulting rod was 41.5 cm high. The open top was sealed with a small piece of masking tape, to protect the paper from the treatments applied to the tubing.

In four preliminary experiments, six coloured rods (blue, green, yellow, red, black, white) were presented in a cylindrical acetate sleeve atop a plastic standard plant pot of 15 cm diameter. Each rod had five large pea aphids ($A. pisum$) smeared onto the top 1/3 with forceps (carcasses removed). Ovipositing female $E. volucris$ were introduced individually so that their behaviour could be continuously observed for two or four hours. On four days, the six coloured rods were painted on the top 1/3 with fluid from 3-400 pea aphids crushed in 2 ml distilled water (carcasses removed) and presented to mixed cages of ovipositing $M. venablesi$ and $E. volucris$ with no other oviposition
stimulus. Rods were removed after 8 h and examined for eggs laid. Following these experiments, pairs of untreated green rods were presented to syrphids of both species in maintenance cages and any activities were recorded for two hours. The types of activities noted were taken to be related to the presence of the vertical rods, not to any chemical stimulus on the glass. Activities related to ovipositional behaviour of female syrphids were observed over periods of several hours after the flies were presented with living oviposition plants.

In all remaining experiments, pairs of rods were introduced to 70 x 60 x 50 cm cages of adult syrphids (M. venablesi and E. volucris) in which the numbers of each sex were approximately equal. Actual numbers, age, ovipositional status, species, and time of day were noted. Observation was continuous for one-half to two hours, but results were usually recorded for a one-hour period. Individual visits were monitored for specific activities and their duration was recorded. Occasionally, when activity was too intense to monitor individuals, counts of numbers, sex, and main activities were made at one-minute intervals. Six hours of observation were devoted to each of the rod conditions unless otherwise noted. Results were expressed in frequency of visits to experimental and control rods. Frequency of visits for individual observation hours within a group were compared by the Chi-squared test (to assess differences in time of day, species, and any individual differences that occurred) before being combined. The Chi-squared test was used within specified confidence limits to determine whether the recorded number of visits to the paired control and treated rods differed significantly from equality. Materials and methods of individual experiments are described below.
Crushed-bean Rods

A young broad bean plant, 15 cm high, with two pairs of leaves, was cut just above soil level and chopped into fine pieces in a small beaker containing 10 ml distilled water. The plant material was then crushed and mixed with the water and painted onto a green rod with a camel-hair brush. The control rod was painted with distilled water applied with another brush. Both rods were removed after 30 minutes of observation and re-painted to keep them wet during the trial.

Tryptophan and Indoleacetaldehyde Rods

Saturated tryptophan (1.18% in water) and 0.5% indoleacetaldehyde solutions were made up with distilled water and stored at 4 degrees C. A green rod was painted with both of these solutions and allowed to dry. The control rod was untreated. After a one-hour observation period, each rod was removed and cleaned.

Honeydew Rods

Rods to be treated were placed for at least 12 hours in a cage of broad beans heavily infested with pea aphids. If a rod was used for two experiments in one day it was returned to the aphid cage between experiments. At the end of each day the treated rod was cleaned. Control rods were untreated.

Dead Aphids Glued to Rods
Pea aphids were killed by freezing. Immediately before the observation period, a green rod was painted in three places with clear nail polish and a thick 3.6 x 1.0 cm mat of aphids was placed on each patch before the nail polish dried. Control rods were treated only with nail polish. Observations were limited to a maximum of one hour to insure that the aphids remained fresh.

Black Spots

Black spots were applied with a washable felt marker to three places on a rod to simulate three "colonies". The size of each colony was 3.6 x 1.0 cm, similar to the patches of dead aphids described above. Control rods were not marked.

Aphids in Glass Vials

A 5 cm glass vial containing 20 pea aphids caged with cotton wool and gauze was attached by an elastic band 10 cm from the top of each rod. The vials were placed perpendicular to the rods. Control vials were empty. This test was based on the assumption that the flies could see the aphids moving about inside the experimental vial, while olfactory and gustatory cues were reduced by the cotton plug.
Results and Discussion

Olfactometer Experiments

1. Air Flow Controls

Air-flow control experiments were conducted on 73 insects, with and without green paper over the mixing zone (55 with, 18 without) and with the stimulus chambers empty. There was no response to either stimulus chamber. In subsequent trials, when one chamber contained a stimulus and the other acted as a blank control, only 2 of 305 (0.66%) insects responded to the control chamber. Only one control test situation gave rise to any response in the absence of olfactory stimuli. Twenty-seven insects were tested with pairs of colours over the mixing zone (green, with yellow or with red). Two females and one male entered the empty stimulus chambers, one per colour, a response frequency of 11.11%. These two response frequencies were used as expected values in the Z tests of the various responses to olfactory stimuli.

2. Colour Over Mixing Zone

Fourteen mated, first generation females previously exposed to an oviposition plant (5 M. venablesi, 9 E. volucris) were exposed to a heavily infested broad bean plant in one of the olfactometer stimulus chambers. Insects were exposed to the stimulus from 2 to 5 1/2 hours with no coloured paper over the mixing zone. None of the insects responded by searching for the ovipositional stimulus. Subsequent to these experiments, green paper was placed over the mixing zone. When the
insects were retested under these conditions they responded to the infested bean plant by searching for and finding their way into the stimulus chamber. This change in their behaviour suggests that, without some visual stimulation, such as the colour of a potential host plant, these females could not or would not respond solely to odours with which they were already familiar. Visual and olfactory cues thus seem to be linked to one another.

3. Real Flower Olfactory Stimuli

All insects used in these trials were from first- or second-laboratory-reared generations. Their ages varied from one day to four weeks, with or without feeding experience on flowers. Two types of flower were employed, mock orange (Philadelphus sp.) with a very strong fragrance, and a mix of garden annuals, including those used in the real-flower preference studies (Appendix 1). The fragrance of this flower mixture was not so strong as the mock orange. Responses were recorded when each flower type was tested against an empty control chamber in Table I.
Table I. Olfactometer Response of Syrphids Exposed to Real Flowers

1. Response to Mock Orange

<table>
<thead>
<tr>
<th>Insects used</th>
<th>Insects responding</th>
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</thead>
<tbody>
<tr>
<td>Species</td>
<td>Sex</td>
</tr>
<tr>
<td>M. venablesi</td>
<td>males</td>
</tr>
<tr>
<td></td>
<td>females</td>
</tr>
<tr>
<td>E. volucris</td>
<td>males</td>
</tr>
<tr>
<td></td>
<td>females</td>
</tr>
</tbody>
</table>

2. Response to Garden Mix

<table>
<thead>
<tr>
<th>Insects used</th>
<th>Insects responding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>Sex</td>
</tr>
<tr>
<td>M. venablesi</td>
<td>males</td>
</tr>
<tr>
<td></td>
<td>females</td>
</tr>
<tr>
<td>E. volucris</td>
<td>males</td>
</tr>
<tr>
<td></td>
<td>females</td>
</tr>
</tbody>
</table>

Totals: males 14 3  
         females 40 7
The total response was 18.5%. The Z test showed that there was a significant response to flowers (P < 0.01 that the response occurred by chance), but that the observed difference in response to different flowers was due to chance. Also, response differences between males and females were not significant; both responded to flower odours to the same degree. Previous experience with flowers did not affect the response.

These trials showed that syrphids respond to the odour of their food source - flowers. Although this response has not been previously reported in the literature, it is an established fact for bees (von Frisch 1969) and these two groups display convergent evolution in a number of traits, including behaviour, appearance and habitat choice (Bishop & Chung 1972). Kaib (1974) showed that there are separate flower odour receptors on the antenna of the calliphorid, C. vicina, used for adult food recognition, whereas the meat odour receptors are used for oviposition site recognition. Kaib's results suggest that syrphids might also use different sensilla to recognize food and oviposition sites.

4. Uninfested Bean Plants; Whole and Crushed

To test for olfactory attractiveness of plants without aphids, one chamber of the olfactometer was charged with a whole broad-bean plant, or one crushed in distilled water was presented on saturated paper tissues ("Kimwipes") in a petri dish. Forty-two mated individuals (11 ♂, and 7 ♀ of E. volucris, and 19 ♂ and 5 ♀ M. venablesi) were tested with the whole plant and 10 (M. venablesi, 5 ♂ and 5 ♀) with the crushed
bean. Control chambers remained empty when the whole plant was used. Water-soaked tissues in a petri dish were the control for the crushed plant.

There was only one response from a female (\textit{E. volucris}) to the control chamber in the crushed bean trials. There was no evidence that either sex of these species would search further in response to the odour of whole or crushed bean plants.

5. Aphids as a Stimulus

Since aphids constitute a major aspect of an oviposition site, groups of aphids were presented in the olfactometer to 10 mated and ovipositing \textit{E. volucris} females. Three to four hundred potato aphids (\textit{Macrosiphum euphorbia}) were placed in a 10 X 12 cm nylon bag and suspended from the ceiling of the test chamber. A control nylon bag was similarly suspended in the control chamber. Since there was no response after two hours, the females were left in the chamber for an additional 1.25 hours. They still did not respond, but half of them laid eggs when tested with aphids in a vial after this experiment was concluded. Either the stimulus in the olfactometer was not sufficiently strong, or aphid odour alone was not enough to elicit search flights for an oviposition site. In \textit{S. luniger}, at least, aphid odours alone were reported to have stimulated oviposition (Dixon 1959).

6. Tryptophan and Indoleacetaldehyde as Olfactory Stimuli
Twenty-two unmated female *E. volucris*, 18 mated females (10 *M. venablesi*, 8 *E. volucris*) and 10 unmated males (*E. volucris*) were tested in the olfactometer with a mixture of 1.18% tryptophan and 0.5% indoleacetaldehyde solutions in distilled water on saturated tissues ("Kimwipes"). The control was water-saturated tissues ("Kimwipes"). The mated females had been exposed to an oviposition plant either 24 or 48 hours before the experiment. Two mated females (one of each species) responded, but no male or unmated female reacted. Five of the mated females which did not respond were then tested with one chamber containing an infested bean plant and one empty chamber. Three (1 *E. volucris*, 2 *M. venablesi*) responded to the infested bean.

Two responses in 18 is significant when tested by the Z test. But the fact that three of five female nonresponders later responded to a real oviposition stimulus suggests that the stimulus from the chemicals in the chamber was relatively weak. Responders were from the 2-week and 3-4 week age groups, while mated nonresponders were either newly emerged or from the 2-week age group. Age might have affected their response. Although there was a demonstrated response, these results are not so striking as those of van Emden and Hagen (1976) with green lacewings. The lacewings were much more responsive during olfactometer trials.

7. Infested Host Plants as Olfactory Stimuli

Flies in several physiological states were tested with the "normal" oviposition stimulus, a broad-bean plant infested with either pea- or black-bean aphids. "Unmated" refers to insects never exposed to the opposite sex. "Mated" refers to insects kept with both sexes in cages
in which matings were observed. "Naive" refers to insects not previously exposed to any part of the ovipositional stimulus. "Experienced" insects are those previously exposed to the oviposition stimulus or some part of it. "Deprived" refers to "experienced" insects not exposed to an oviposition plant in their cages for 24 or 48 hours prior to testing.

7.1. Unmated Naive Insects: Never Exposed to Aphids or Plants

Fifty-five unmated females (43 M. venablesi, 12 E. volucris) and 4 unmated males (3 M. venablesi, 1 E. volucris), all less than one week old, were tested with a heavily infested bean plant in the olfactometer. Two female M. venablesi responded. This response is significant (P<0.01, Z test) suggesting that there is some innate response to oviposition stimuli even before there is a physiological need to oviposit. The few males tested did not respond.

7.2. Unmated Experienced Insects: Previously Exposed to Plants and Honeydew, but not Aphids

Ten female and 19 male, unmated E. volucris were exposed in the olfactometer to air passed over a broad-bean plant infested with pea aphids. These insects had never been exposed to aphids, but had been exposed to a bean plant with honeydew in their cage prior to the experiment. Two females and 4 males responded to the stimulus. Although sample sizes were too small to compare the sexes, the Z test of the combined response was significant (P<0.01). This result shows that previous exposure to at least part of the stimulus improves recognition of the stimulus, even before there is a physiological need to oviposit.
For males, the recognition would not have been ovipositional, but was probably in response to odours associated with a food source (honeydew). Females might have been responding to the food source, the ovipositional cues, or both.

7.3. Mated Naive Females: Never Exposed to Aphids or Plants

Nine mated *E. volucris* females were exposed to an infested broad-bean plant in the olfactometer. There was no green paper over the mixing zone. Five mated *M. venablesi* females were similarly exposed to an oviposition plant, but the mixing zone was covered with green paper. No response occurred in the absence of green paper as one would expect from earlier preliminary experiments. One insect responded when the green paper was present. Though the sample size is small, one response out of five is significant (*P* < 0.01, Z test).

Because even unmated flies responded differently after prior exposure to host plants and honeydew, mated and ovipositing females were tested along with their respective males in the next set of experiments, after they had had varying previous experience with host factors.

7.4. Mated Experienced Insects: Oviposition Plant in Cage Immediately Prior to Experiment

Forty-five mated females (18 *M. venablesi*, 27 *E. volucris*) were removed from a cage containing an oviposition plant and tested in the olfactometer with a similar plant. Thirteen insects (8 *M. venablesi*, 5 *E. volucris*) responded to this stimulus, a significant response (*P* < 0.01, Z test).
Twenty-nine mated *E. volucris* males were also tested in a similar sequence. Their lack of response was unexpected, since unmated males had responded to the same stimulus after previous exposure to it.

7.5. Experienced Insects: Oviposition Plant Deprived- 24 or 48 Hours

Thirty-five mated and oviposition plant-deprived females (25 *M. venablesi*, 10 *E. volucris*) and ten similarly conditioned *M. venablesi* males were exposed to an oviposition plant in the olfactometer. Three male and five female *M. venablesi* responded, a significant result (*P* < 0.001, Z test). Oviposition plant deprivation thus may increase the sensitivity of these insects or lower their threshold for searching behaviour.

The following contingency table illustrates the different responses of ovipositing females and mated but non-ovipositing females not previously exposed to an oviposition plant.

<table>
<thead>
<tr>
<th>NO RESPONSE</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>not depr.</td>
<td>deprived</td>
</tr>
<tr>
<td>ovipositing females</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>nonovipositing females</td>
<td>22</td>
<td>21</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>RESPONSE</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>not depr.</td>
<td>deprived</td>
</tr>
<tr>
<td>ovipositing females</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>nonovipositing females</td>
<td>6</td>
<td>2</td>
</tr>
</tbody>
</table>
Chi-squared tests of pairwise interactions with the computer programme NED, 3WAY (UBC Computing Center Document 13.2), showed a significant interaction between ovipositing and non-ovipositing females and their respective responses. There was a significant ($P<0.01$, Chi-squared test) relationship between the ovipositional status of the female and the degree of response. On the other hand, there was no significant response related to plant deprivation. Since the 1-2 day period of oviposition-plant deprivation was rather short in relation to the lifespan of the insects, a longer deprivation period possibly might have had more effect.

Table II lists the indices of attractiveness calculated for each of the olfactory stimuli (species combined) by the following equation:

\[
\text{INDEX} = \frac{\text{number responding to stimulus}}{\text{in outer chamber} + \text{in stimulus chamber} + \text{in control chamber}}
\]

Apart from the oviposition plant, mock orange was the most attractive stimulus. The insects most sensitive to the oviposition plant were mated experienced females and mated deprived males. Although the mated experienced females were responding to ovipositional stimuli, the males were probably only responding to an odour they had learned to associate with food (in the form of honeydew). In any event, the results show that males, besides possessing the same antennal sensilla types as females, are capable of responding to at least some of the olfactory cues to which females respond.
Table II. Attractiveness Indices for All Stimuli Presented to Syrphids in the Olfactometer

<table>
<thead>
<tr>
<th>STIMULUS</th>
<th>INDEX</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Flowers</td>
<td></td>
</tr>
<tr>
<td>mock orange</td>
<td>0.280</td>
</tr>
<tr>
<td>garden mix</td>
<td>0.103</td>
</tr>
<tr>
<td>B. Others</td>
<td></td>
</tr>
<tr>
<td>bean plant</td>
<td>0.000</td>
</tr>
<tr>
<td>crushed bean</td>
<td>0.000</td>
</tr>
<tr>
<td>aphids</td>
<td>0.000</td>
</tr>
<tr>
<td>tryptophan/indoleacetaldehyde</td>
<td></td>
</tr>
<tr>
<td>unmated females</td>
<td>0.000</td>
</tr>
<tr>
<td>mated females</td>
<td>0.111</td>
</tr>
<tr>
<td>C. Oviposition Plant Offered to;</td>
<td></td>
</tr>
<tr>
<td>unmated naive females</td>
<td>0.036</td>
</tr>
<tr>
<td>unmated experienced males</td>
<td>0.211</td>
</tr>
<tr>
<td>unmated experienced females</td>
<td>0.200</td>
</tr>
<tr>
<td>mated naive females</td>
<td>0.200</td>
</tr>
<tr>
<td>mated experienced females</td>
<td>0.289</td>
</tr>
<tr>
<td>mated deprived females</td>
<td>0.139</td>
</tr>
<tr>
<td>mated deprived males</td>
<td>0.300</td>
</tr>
</tbody>
</table>
Oviposition Rod Experiments

Preliminary Experiments

In four trials of individual first-generation *E. volucris* exposed to six coloured glass rods smeared with pea aphids, none of the females laid eggs or showed any recognition of the rods as an ovipositional stimulus. Because of the absence of response in these females, similarly treated sets of rods in six colours were presented to first-generation ovipositing females of both species in their maintenance cages. In four days of exposure and observation, no eggs were laid on the glass rods, nor did syrphids spend any time searching the rods.

These results do not match Dixon's (1959) findings that such treated rods induced oviposition in *S. corollae*. In fact, in Dixon's experiments, green rods promoted significantly more laid eggs than did white or black. This could be a species difference or perhaps might be an effect of laboratory rearing on *S. corollae*. The two species studied in my experiments were never more than one generation removed from the wild state. *S. corollae*, on the other hand, is easily reared in the laboratory and thus is commonly kept in laboratories for many generations (see refs. in Schneider 1969). There is no information in Dixon's (1959) paper regarding the number of generations her stock of *S. corollae* had been in the laboratory.

Individual flies in a cage of ovipositing *M. venablesi* and *E. volucris* made 47 visits to two green glass rods during two hours. Activities observed included landing without hovering (18), hovering then landing (5), landing on top (27), fast walking (11), preening (9),
and resting (38). Two visits also included tasting. These activities were assumed to be related to the presence of vertical glass rods, not to sources of food or oviposition sites.

In addition to these activities, the following responses also occurred on real oviposition plants: (a) the substrate was tasted with the labellum and this tasting was accompanied by slow searching and frequent turning; (b) prior to oviposition, the abdomen was frequently wagged up and down while the ovipositor was extended and dragged on the substrate. These additional, and more intense activities thus could be related to specific ovipositional stimuli in the oviposition sequence.

All these activities could be divided into three categories: activity prior to and including landing; activity related to the stimulus; and unrelated activity. In the first category, recorded activities included:

- hovering with landing
- hovering without landing
- landing without hovering

In the second category, activities recorded included:

- repeated take-off with hovering and landing again
- tasting
- walking slowly
- turning
- abdominal waggling
- ovipositor extension
- egg laying
Recorded activities unrelated to the stimulus included:

- landing on top
- walking fast
- preening
- resting

On rods in preliminary experiments, and on treated rods in subsequent experiments, the activities noted were interpreted as follows. Hovering without landing indicated some recognition, either visual or olfactory, of the object being investigated. Landing after hovering was the next step in the attraction of insects to a rod. Repeated take-offs with hovering and landing occurred when the stimulus on the rod attracted a large number of insects, and thus it could be related to the degree of excitation generated by the stimulus. Landing without hovering was the most common means of settling on a rod, but where the landing took place on the rod was not always related to the treatment. For example, landing on top of the rod was usually associated with resting and preening behaviour and was rarely followed by investigation of the rest of the rod. Thus, landing on the top was primarily a response to a choice perching position. Males landed on top most frequently, and would often challenge each other for a resting spot on the top of the rod.

"Tasting" referred to persistent labellar contact with the rod lasting longer than two seconds. Females were more likely than males to include tasting in their visits, and the most frequent or persistent tasting was associated with the rods that received the most visits. Tasting seemed to be associated with the previous treatment of the rod.
Walking slowly, with turns, usually accompanied tasting and seemed to be part of the searching sequence initiated by tasting. Walking fast usually occurred during short visits, and was not always associated with the stimulus on the rod. The speed of searching might be modified on a treated rod after some apparent recognition (by tarsal gustation, olfaction, vision, or tasting). Without this apparent recognition, some unrelated behaviour, such as preening or resting, followed.

Abdominal movements (waggling) only accompanied tasting with slow turning and thus probably indicated a further increase in response to the stimulus. Ovipositor extension and egg laying were the culmination of the sequence.

Preening (unless it involved the ovipositor after egg laying) and resting were non-specific activities unrelated to the stimulus, as were landings on top of the rod. They tended to occur on control rods as frequently as on experimental rods.

The experiments in which pairs of green glass rods were presented to syrphids were grouped for comparison into four categories, depending on the sensory cues available:

A. Visual Colour Preference
   1. green vs yellow rods (both untreated)
   2. green vs yellow rods (both honeydew treated)

B. Gustatory and Olfactory Stimuli without Visual Cues
   1. crushed broad-bean vs water control
   2. tryptophan and indoleacetaldehyde vs water control
   3. honeydew vs untreated control
   4. tryptophan and indoleacetaldehyde vs honeydew
C. Visual Stimuli with Limited or without Gustatory or Olfactory Cues

1. black marker spot "colonies" vs untreated control
2. live aphids in a glass vial vs empty vial control

D. Visual, Gustatory and Olfactory Cues

1. dead aphids glued to rod vs glue control
2. dead aphids glued to rod vs honeydew

In experiments with identical conditions, Chi-squared analysis revealed that there were no differences between the frequencies of morning and afternoon visits among males, females, or the sexes combined. Nor were there any significant differences in duration of visits (P<0.05, analysis of variance) for either sex in morning or afternoon. For these reasons, experiments with identical rod conditions were grouped together and the combined results are reported here.

1. Activities Unrelated to the Stimulus

As expected, activities not related to the stimulus on a rod did not vary from experimental to control rods, except in two cases. These both involved insects doing more fast walking (Chi-squared, P<0.001), once on a tryptophan- and indoleacetaldehyde-treated rod (vs honeydew) and once on a control rod (3 colonies of fresh dead aphids vs control). In both cases, females included fast walking in their visits more frequently than did males. This difference did not occur in any other experiment. Unrelated activities will not be discussed further.

2. Visual Colour Preference
2.1. Green vs Yellow Rods (both untreated)

Observations on each species included one hour in the morning and another in the afternoon. Chi-squared analysis showed that there was no difference in the frequency of visits to yellow (21) and green (19) rods.

Figure 1 shows the frequency of stimulus-related activities on each colour. In these experiments, most activity was of the nonspecific sort, and there was no preference for either rod in any activity.

2.2. Green vs Yellow Rods (both honeydew treated)

Observations included three and one-half hours in the morning and two and one-half hours in the afternoon. Two of the morning hours were with E. volucris, and the remainder with M. venablesi. All afternoon observations used M. venablesi. Visit duration for males on the yellow rods was shorter but not significantly so, than that on the green rods.

Figure 2 shows the frequency of all stimulus-related activities on each colour. Most activity involved landing, tasting, slow searching with turns, and repeated take-offs with hovering and further landings. Each colour had the same level of activity. There was no hovering without landing on either colour, indicating little if any visual interest.
Figure 1. Syrphid Activity on Untreated Green and Yellow Glass Rods

Frequency of landing (a) and stimulus-related activities (b) observed in 2 h of study with both sexes of *M. venablesi* and *E. volucris* presented with two untreated glass rods, one green and one yellow.

Legend

a.  1  hovering without landing
    2  hovering with landing
    3  landing without hovering

b.  1  repeated take-offs with hovering and landing
    2  tasting
    3  slow walking
    4  with turning
    5  abdominal waggling
    6  ovipositor extension
Figure 2. Syrphid Activity on Honeydew-Treated Green and Yellow Glass Rods

Frequency of landing (a) and stimulus-related activity (b) observed in 5 h of study with both sexes of *M. venablesi* and *E. volucris* presented with two honeydew-treated glass rods, one green and one yellow.

**Legend**

a. 1 hovering without landing
2 hovering with landing
3 landing without hovering

b. 1 repeated take-offs with hovering and landing
2 tasting
3 slow walking
4 with turning
5 abdominal waggling
6 ovipositor extension
In these experiments, colour by itself was not important for either species, whether or not it was associated with food (honeydew). When both rods were treated with a substance containing feeding as well as ovipositional stimuli, there was some indication that visit duration might have decreased slightly for males on the yellow rod, but there was still no overall significant difference. Since honeydew may be present on both green and yellow plants, one would expect the flies to have no marked colour preference while foraging. In these experiments, the insects accepted food wherever they found it. This result differs from the previously noted attraction of some syrphid species to yellow (Dixon 1959; Ilse 1949; Peschken 1965). Dixon (1959) concluded that yellow flowers, by virtue of their colour, could be more attractive to syrphids than other colours. In the present experiments, yellow rods, by virtue of their colour alone, were not more attractive than green, with or without association with feeding or ovipositional cues. My results are also contrary to those obtained for S. corollae (Bombosch & Volk 1966) in that M. venablesi and E. volucris would not oviposit on honeydew without other inducement.

3. Gustatory and Olfactory Stimuli without Visual Cues

3.1. Crushed Broad Bean vs Distilled Water Control

All insects were third- and fifth-generation lab-reared M. venablesi. Observations included three morning and four afternoon hours. There was a significant ($P<0.001$, Chi square) preference for crushed bean, as can be seen in the following table:
<table>
<thead>
<tr>
<th></th>
<th>control</th>
<th>crushed bean</th>
</tr>
</thead>
<tbody>
<tr>
<td>male visits</td>
<td>80</td>
<td>128</td>
</tr>
<tr>
<td>female visits</td>
<td>64</td>
<td>142</td>
</tr>
</tbody>
</table>

A significantly greater number of insects (Figure 3) landed on the treated rods, repeatedly took-off, then hovered and landed, and waggled their abdomens up and down. Females visited the experimental rods more often than males, and tasted and walked slowly and turned more often. Even on the control rods, females tasted significantly more often than males.
Figure 3. Syrphid Activity on Green Glass Rods; Crushed Broad-bean Treated vs Untreated Control

Frequency of landing (a) and stimulus-related activities (b) observed in the 7 h when males and females of *M. venablesi* and *E. volucris* were presented with two green glass rods, one untreated control and one treated with crushed broad-bean liquid. Significant differences (P<0.001, Chi square) between control and treated rods are indicated by an asterisk above the "treated" column.

Legend

a. 1 hovering without landing
    2 hovering with landing
    3 landing without hovering
b. 1 repeated take-offs with hovering and landing
    2 tasting
    3 slow walking
    4 with turning
    5 abdominal waggling
    6 ovipositor extension
    7 oviposition
Both males and females showed much more interest in the crushed-bean than in the control rod, but females tended to taste and search longer on this treated rod. Although a crushed plant does not the same stimulus as an intact plant, the sex differences in response during these trials indicate that plant tastes or odours are involved in the oviposition sequence. Considering the negative results obtained in olfactometer trials with both whole and crushed bean, I suggest that the gustation of the host plant is important in selecting an oviposition site. Host-plant odour alone will not initiate search flights for oviposition sites. On the other hand, the taste of a crushed host plant on an artificial substrate will initiate slow methodical searching involving a number of the elements of an ovipositional site search (i.e. stimulus related activities).

3.2. Tryptophan and Indoleacetaldehyde vs Untreated Control

In these trials, females were observed for five and one quarter hours (2.5 morning and 2.75 afternoon) and males were watched for one hour. Since females of *M. venablesi* and *E. volucris* shared the same cages, the results for females were combined. The results also showed that the frequency and duration of visits by older insects (2-3 weeks), did not differ from those by insects 1-2 weeks old. There were 39 visits to the control rods and 64 to the treated rods, but Chi-squared analysis showed no significant difference (P>0.10) between them.

Figure 4 shows the frequency of each stimulus related activity during female visits. Treated rods showed consistently more of each type of specific activity. Only in slow walking, however, were the
differences sufficiently great to be statistically significant (P < 0.001).
Figure 4. Female Syrphid Activity on Green Glass Rods; Tryprophan and Indoleacetaldehyde Treated vs Water Control

Frequency of landing (a) and stimulus-related activities (b) observed in 5.5 h of study with females of *M. venablesi* and *E. volucris* presented with two green glass rods, one untreated and one treated with tryptophan and indoleacetaldehyde.

Legend

a. 1 hovering without landing  
   2 hovering with landing  
   3 landing without hovering  

b. 1 repeated take-off with hovering and landing  
   2 tasting  
   3 slow walking  
   4 with turning  
   5 abdominal wagging  
   6 ovipositor extension
During the one-hour observation on males there were only four visits to each rod. There were two nonspecific top landings on the experimental rod. Only one of the other two visits to this rod involved tasting, which was not followed by searching. The remaining visits to control and experimental rods all involved nonspecific activities, such as preening, walking fast or resting.

Differences were not so striking between control and treated rods in this experiment, but females did visit the treated rod more than the males (averaging 19 visits per hour vs the males' 4 per hour) and spent their time there in stimulus-related activity. Since the chemicals used in this treatment are important components of the aphid product, honeydew, the preference females displayed for the treated poles is explicable in terms of host-finding behaviour. The results of these experiments confirm the attraction, however slight, for these chemicals observed in olfactometer trials. The work of Hagen et al. (1976) and van Emden and Hagen (1976), on the attraction of these chemicals for green lacewings suggested this experiment. Syrphids seem sensitive to at least some of the same components of honeydew that attract lacewings. This should not be surprising in view of the ecological similarities between the two kinds of insects.

3.3 Honeydew vs Untreated Control

Cages containing equal numbers of both sexes of M. venablesi and E. volucris were observed for three morning and three afternoon hours. During half of these experiments, an oviposition plant infested with pea aphids remained in the cage, while the other half did not include a
plant. The presence of the plant made no statistical difference to the responses of males or females to the glass rods. All observations therefore could be combined.

Both sexes visited the honeydew-treated rod more often than the control ($P < 0.001$). Actual frequencies of visits to each rod are as follows:

<table>
<thead>
<tr>
<th></th>
<th>honeydew</th>
<th>control</th>
</tr>
</thead>
<tbody>
<tr>
<td>male visits</td>
<td>73</td>
<td>40</td>
</tr>
<tr>
<td>female visits</td>
<td>74</td>
<td>27</td>
</tr>
</tbody>
</table>

Figure 5 shows the frequency of stimulus related activities on each rod. The following activities occurred with significantly greater frequency on the honeydew-treated rods: landing without hovering; repeated take-offs with hovering and landing; tasting; walking slowly. Mean duration of visits to the treated rod was not longer than the average visit to the control rod, because repeated take-offs with hovering before subsequent landings inevitably produced shorter, though more frequent, visits.
Figure 5. Syrphid Activity on Green Glass Rods;
Honeydew-Treated vs Untreated Control

Frequency of landing (a) and stimulus-related activities (b) observed during the 6 h of study in which males and females of M. venablesi and E. volucris were presented with two green glass rods, one untreated and one treated with aphid honeydew. Significant differences between control and treated rods are indicated by an asterisk above the treated column.

**Legend**

a. 1. hovering without landing
2. hovering with landing
3. landing without hovering

b. 1. repeated take-offs with hovering and landing
2. tasting
3. slow walking
4. with turning
5. abdominal waggling
6. ovipositor extension
Honeydew was very attractive to both males and females. As in experiments with green and yellow honeydew-treated rods, no oviposition occurred, confirming that honeydew alone is not sufficient stimulus to induce oviposition in \textit{M. venablesi} or \textit{E. volucris}. There is a high proportion of sugar in honeydew (Schneider 1969) which would provide an attractive food source for both sexes, and it is a known food source for syrphids (Schneider 1969). In effect, male visits provided additional data for assessing the ovipositional attraction of honeydew for females. For example, if more females than males were attracted to a rod that had ovipositional as well as feeding attractants, then the surplus could be attributed to the ovipositional attractant. In these experiments, females visited the treated rod as often as males but visited the control rod only half as often as males. In other words, they favoured the honeydew rod more than the males did. The next step therefore would be to test each of these stimuli on separate rods presented simultaneously to both sexes. The next set of experiments provided such a test.

3.4 Honeydew vs Tryptophan and Indoleacetaldehyde Treated

Observations included two morning hours with both sexes of \textit{E. volucris}, and one afternoon hour with both sexes of \textit{M. venablesi}.

There was no sex difference in total visits among the treatments (HD 81, T&I 79). Furthermore, neither males alone nor females alone showed any preference for either treatment. On tryptophan- and indoleacetaldehyde- treated rods, however, there were significantly more female than male visits ($P < 0.001$ Chi-square). No such difference occurred on honeydew-treated rods.
Figure 6. Syrphid Activity on Green Glass Rods; Honeydew-Treated vs Tryptophan and Indoleacetaldehyde Treated

Frequency of landing (a) and stimulus-related activities (b) observed in the 3 h during which male and female *M. venablesi* and *E. volucris* were presented with two green glass rods, one honeydew-treated and the other treated with tryptophan and indoleacetaldehyde.

Legend

a. 1 hovering without landing  
    2 hovering with landing  
    3 landing without hovering  

b. 1 repeated take-offs with hovering and landing  
    2 tasting  
    3 slow walking  
    4 with turning  
    5 abdominal waggling  
    6 ovipositor extension
Figure 6 shows the frequency of stimulus-related activity on both rods. Significantly more slow walking and turning occurred on honeydew-treated rods ($P < 0.001$). Females on tryptophan and indoleacetaldehyde rods engaged in significantly more landings without hovering, and also tasted significantly more often than males ($P < 0.001$).

The sex difference in the response to the tryptophan- and indoleacetaldehyde-treated rods is good evidence that tryptophan and/or indoleacetaldehyde are gustatory steps in the sequence of stimuli that readies a female for oviposition. The earlier evidence from olfactometer trials indicated that there was also an olfactory response which, however, was far from maximal. The gustatory response to these treated glass rods was far stronger. Labellar gustation of honeydew and its components was previously suggested by Chandler (1966) but to date there was no experimental evidence. Dixon (1959) reported proboscis extension after alighting, but without the proboscis or ovipositor touching the substrate. My results show that labellar gustation may often be a major event in the sequence during which the ovipositional attractants, tryptophan and indoleacetaldehyde, in honeydew are recognized by a searching female.

4. Visual With Limited or No Gustatory or Olfactory Cues

4.1. Black Marker Spot "Colonies" vs Untreated Control

There were three hours of morning and three hours of afternoon observation of control and black-spotted rods. Two hours in each period were devoted to the females of both species, while the sexes were mixed
for the remainder of the observations. One morning experiment was done in the presence of an oviposition plant, but there was no difference in the frequency of visits to the rods, whether or not plants were present. Since the species were combined in cages, no species difference was considered. When overall frequency of visits to control and black spotted rods were compared, there was no significant difference between the sexes.

Figure 7 shows the frequency of stimulus related activities on each rod. The most frequent activities on both rods were the non-specific ones. Since there was nothing but aphid-like spots to elicit slow searching, tasting, or abdominal waggling, such behaviour was rare. Note, however, that most of these activities occurred on the "spotted" rod. Very different behaviour occurred at the spots. Fourteen of seventeen cases of hovering without landing occurred in front of the spots. Of the 25 landings without preliminary hovering on the spotted rod, 15 were directly onto spots. Landings without hovering were significantly more frequent on the spotted rod than the control (P<0.001, Chi-square).
Figure 7. Syrphid Activity on Green Glass Rods; Black Marker Spot-Treated vs Untreated Control

Frequency of landing (a) and stimulus-related (b) activities in the 6 h during which male and female M. venablesi and E. volucris were presented with two green glass rods, one untreated and one marked with 3 "colonies" of black marker spots that simulated aphids.

Legend

a. 1 hovering without landing
    2 hovering with landing
    3 landing without hovering
    4 hovering at spots without landing
    5 landing on spots

b. 1 repeated take-offs with hovering and landing
    2 tasting
    3 slow walking
    4 with turning
    5 abdominal waggling
    6 ovipositor extension
This treatment did not elicit the same type of response as the taste or odour experiments. The visual responses to the spots were unique to this experiment. The considerable amount of hovering in front of the spots indicated visual attraction. Many more insects landed on the spotted than on the control rod, but a lack of gustatory and/or olfactory cues after landing cut short the searching sequence. Even so, it is noteworthy that females visited longer than males (e.g. females 6.8 min. ±12.2, males 1.9 min. ± 4.2). Females were more persistent in following cues not directly associated with food; i.e. patterns of spots on a green stem-like background. These results confirm the observation of Chandler (1968b) that female syrphids were attracted to an optical pattern resembling aphids, especially if the colour contrasted with the plant. In close-range searching, therefore, visual cues of patterns resembling aphids on stems are important to the ovipositional sequence. These results are contrary to Dixon's (1959) conclusion that visual perception of aphids is of little, if any importance, to ovipositing aphidophagous syrphids.

4.2. Live Aphids in a Glass Vial vs Empty Vial Control

This experiment was designed to test the visual response to live, moving aphids without their normal odours. Both sexes of E. volucris were observed for one hour in the morning and one hour in the afternoon. There was no difference in the numbers of male or female visits to the control or experimental rods.
Figure 8. Syrphid Activity on Green Glass Rods;

Live Aphids in an Attached Vial vs Empty Vial

Frequency of landing (a) and stimulus-related (b) activities observed during 2 h while male and female M. venablesi and E. volucris were presented with two green glass rods with attached clear glass vials, one empty and the other containing live pea aphids.

Legend

a. 1 hovering without landing
2 hovering with landing
3 landing without hovering
4 land on vial
b. 1 repeated take-offs with hovering and landing
2 tasting
3 slow walking
4 with turning
5 abdominal waggling
6 ovipositor extension
Figure 8 shows the frequency of stimulus-related activities on each type of rod. Most activity was nonspecific. There was no significant difference for any activity between experimental and control rods. Landing on vials was noted separately from landing on rods, but aphids in the vial had no significant effect either. In fact, more insects landed on the control vial than on the vial with aphids.

Vials full of live aphids did not attract males or females. The flies gave no sign that they could recognize aphids in these containers. In fact, the marker spots drew more attention than did the real aphids "out of context". This result, in contrast to the previous experiments, seemed to indicate that Dixon (1959) was correct in assuming that visual perception of aphids was unimportant to the ovipositional sequence of aphidophagous syrphids. In combination with my foraging results, however, this present result suggests that, even if visual perception of aphids alone may not be a sufficient stimulus, the addition of a green stem behind the pattern of aphids makes the pattern recognizable to females searching for oviposition sites.

5. Visual With Gustatory and Olfactory Cues

5.1. Dead Aphids Glued to a Rod vs Control (glue)

Although "colonies" of fresh dead aphids were the closest laboratory approximation to a real oviposition stimulus, dead aphids could not produce honeydew. Consequently most of the taste/olfactory attractants in honeydew were absent from these tests. The following tests of both sexes of the two species occupied five hours in the afternoon and one-
half hour in the morning. A total of 154 males and 152 females were observed. As before, half of the experiments were conducted with an oviposition plant in the cage and the other half without one. There was no significant difference in the responses to the glass rods with or without the oviposition plant. Because there was no difference, all hours of observation could be combined.

Control rods received fewer (65) visits than aphid rods (89), but this difference was not significant. Although females visited both rods more often than males, this difference was significant only on the aphid rods ($P < 0.001$ Chi-square).

Figure 9 shows the frequency of stimulus-related activities on each type of rod. Significantly more syrphids hovered, then landed, on the aphid rods than on the control rods. Other activities that occurred significantly more often on treated rods were, slow walking and turning, and abdominal waggling. Ovipositor extension and egg laying only took place on the "aphid" rods.
Figure 9. Syrphid Activity on Green Glass Rods; Dead Aphids Glued to a Rod vs Glue Control

Frequency of landing (a) and stimulus-related (b) activities observed in the 5.5 h when male and female *M. venablesi* and *E. volucris* were presented with two green glass rods, one glue-control and one with three "colonies" of freshly killed aphids glued to it.

Legend

a. 1 hovering without landing  
    2 hovering with landing on aphids  
    3 landing without hovering  
    4 landing without hovering on aphids  

b. 1 repeated take-offs with hovering and landing  
    2 tasting  
    3 slow walking  
    4 with turning  
    5 abdominal waggling  
    6 ovipositor extension  
    7 oviposition
On rods with freshly killed aphids, females displayed most of the behavioural sequence during which they locate an oviposition site and begin to lay eggs. Even though most individuals seemed disturbed by the substrate, occasionally one would lay an egg, but most probing with the ovipositor was not followed by egg laying. Since the surface texture of smooth glass or nail polish differs greatly from the surface of a leaf, it must differ even more from a leaf surface coated with honeydew. After allowing for such differences in texture, however, the gustatory sensilla on the ovipositor might still have been the major limiting factor, because there was little, if any, honeydew on the glass that held the dead aphids. The fact that some eggs were laid directly on the dead aphids suggests that gustatory stimuli were indeed the most important limiting factor for most of these caged females. Honeydew-contaminated aphids seem to have provided a sufficient stimulus to satisfy the egg-laying requirements of a few of these females.

One important finding in these experiments is that aphids alone, without their host plant could release oviposition. The species investigated here belong to the group for which the aphid is the paramount stimulus (Chandler 1968a). I must disagree with Chandler's (1966) suggestion, however, that the visual stimulus of aphid appendage movement is a prerequisite for oviposition. Also, I would add to the gustatory capacities of the ovipositor sensilla of these species, the ability to recognize the release chemicals in honeydew as well as aphids.

5.2. Dead Aphids Glued to a Rod vs Honeydew Treatment
There were three morning and three afternoon hours of observation of second- and fifth-generation lab reared *M. venablesi* adults exposed to this combination of rods. Cages contained between 13 and 31 males and 14 and 30 females but there were equal numbers (+ or - 1) of each sex in each cage. All insects were two weeks old or less.

Honeydew-treated rods received significantly (P < 0.001 Chi-square) more visits (283) than did aphid rods (195). Males, however, showed a significant preference for honeydew rods, whereas females visited each type with the same frequency. Figure 10 shows the frequency of stimulus-related activities on each type of rod. There were no significant sex differences in any stimulus-related activity on the honeydew rods. On aphid rods, however, males hovered without landing (both at the rod and above aphids) significantly more often than females (Chi-squared, P < 0.001). In all other activities related to the stimulus, females showed significantly (P < 0.001, Chi-squared) greater activity than males.

As expected, there were also great differences between rods. In all landing and stimulus-related activities, honeydew and aphid rods attracted significantly different amounts of activity (Chi-squared, P < 0.001 for all). Aphid rods had more hovering, with and without landing, and this activity was focused on places where there were aphids. Ovipositor extension and attempted oviposition occurred more frequently on aphid rods.
Figure 10. Syrphid Activity on Green Glass Rods:

Dead Aphids Glued to a Rod vs a Honeydew-Treated Rod

Frequency of landing (a) and stimulus-related (b) activities observed during the 6 h of observation in which male and female M. venablesi and E. volucris were presented with two green glass rods, one treated with honeydew and one with three "colonies" of freshly killed aphids glued to it.

Legend

a. 1 hovering without landing
    2 hovering with landing
    3 landing without hovering
b. 1 repeated take-offs with hovering and landing
    2 tasting
    3 slow walking
    4 with turning
    5 abdominal waggling
    6 ovipositor extension
    7 oviposition
Successful oviposition occurred only on aphid rods. In contrast, honeydew-treated rods received more visits involving landing without hovering, repeated take-offs with hovering and landing, tasting, slow walking, turning, and abdominal waggling. In part these increases reflect the greater number of visits to the honeydew.

Females in this test displayed the same behavioural sequence in response to the freshly killed aphids as in the last set of observations with dead aphids on rods. Some females succeeded in laying eggs, although not without some confusion when the ovipositor probed the substrate. The fact that males preferred honeydew rods, whereas females did not, once again provided a "control" for assessing the feeding stimulus that honeydew provided. Males were not so attracted to aphid rods, and their activities on each type of rod differed greatly. Stimulus-related activity that occurred frequently on one rod occurred only infrequently, if at all, on the other. For example, the visual importance of aphids was apparent in the hovering responses to the aphid rod, whereas there was virtually no hovering before landing on the honeydew rod.

In the following three tables, male and females responses to all pairs of rods are summarized. The percentage of visits that included each activity is given for each experiment. In landing activities (Table III), most landings occurred without hovering. Unrelated activities (Table IV) showed no particular trend in sex differences in relation to rod treatment. Stimulus-related activities (Table V) on honeydew rods were high for both sexes. Male and female differences on the other rods are apparent in the tabulated percentages compared.
Table III. Percentage of visits to glass oviposition rods that included specific landing activities

<table>
<thead>
<tr>
<th>Experiment</th>
<th>No. hours</th>
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<th>hover w/o land</th>
<th>land w/o hover</th>
<th>H. on spots</th>
<th>L. on spots/ vial</th>
<th>total visits</th>
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<td>61</td>
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* females only used, "male" column is *M. venablesi* and "female" column is *E. volucris.*
Table IV. Percentage of visits to glass oviposition rods that included specific stimulus-related activities

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<tr>
<th>Experiment</th>
<th>Stimulus-Related Activity</th>
<th>repeated TH&amp;L</th>
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<th>slow walking</th>
<th>turning</th>
<th>abdo. ovi. ext.</th>
<th>eggs &amp; attempts</th>
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<td>Aphid/Vial</td>
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</table>

* females only used, "male" column is *M.* venablesi and "female" column is *E.* volucris.
Table V. Percentage of visits to glass oviposition rods that included activities unrelated to the stimulus

<table>
<thead>
<tr>
<th>Experiment</th>
<th>land on top</th>
<th>walk fast</th>
<th>preen</th>
<th>rest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>m  f</td>
<td>m  f</td>
<td>m  f</td>
<td>m  f</td>
</tr>
<tr>
<td>Colour pref.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. green</td>
<td>58 57</td>
<td>33 29</td>
<td>25 42</td>
<td>33 42</td>
</tr>
<tr>
<td>vs yellow (untreated)</td>
<td>37 30</td>
<td>25 30</td>
<td>37 46</td>
<td>37 8</td>
</tr>
<tr>
<td>B. green</td>
<td>1 5</td>
<td>4 5</td>
<td>5 6</td>
<td>8 13</td>
</tr>
<tr>
<td>vs yellow (honeydew)</td>
<td>0 1</td>
<td>1 2</td>
<td>6 7</td>
<td>16 6</td>
</tr>
<tr>
<td>Gust. &amp; Olfact.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. crushed bean</td>
<td>36 6</td>
<td>11 18</td>
<td>24 6</td>
<td>25 3</td>
</tr>
<tr>
<td>vs water</td>
<td>75 17</td>
<td>6 45</td>
<td>45 41</td>
<td>58 25</td>
</tr>
<tr>
<td>B. Trypto./Indole.*</td>
<td>17 6</td>
<td>32 40</td>
<td>17 26</td>
<td>6 13</td>
</tr>
<tr>
<td>vs water</td>
<td>21 29</td>
<td>32 86</td>
<td>29 43</td>
<td>21 57</td>
</tr>
<tr>
<td>C. honeydew</td>
<td>5 8</td>
<td>8 1</td>
<td>18 19</td>
<td>11 11</td>
</tr>
<tr>
<td>vs control</td>
<td>25 7</td>
<td>28 33</td>
<td>18 15</td>
<td>38 52</td>
</tr>
<tr>
<td>D. Trypto./Indole.</td>
<td>20 19</td>
<td>30 63</td>
<td>25 15</td>
<td>25 9</td>
</tr>
<tr>
<td>vs honeydew</td>
<td>3 8</td>
<td>21 2</td>
<td>14 6</td>
<td>14 13</td>
</tr>
<tr>
<td>Visual</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. black spots</td>
<td>31 19</td>
<td>4 31</td>
<td>35 47</td>
<td>35 41</td>
</tr>
<tr>
<td>vs control</td>
<td>38 58</td>
<td>23 18</td>
<td>62 41</td>
<td>23 45</td>
</tr>
<tr>
<td>B. aphids vial</td>
<td>11 11</td>
<td>56 44</td>
<td>44 56</td>
<td>11 78</td>
</tr>
<tr>
<td>vs vial</td>
<td>0 0</td>
<td>29 36</td>
<td>43 36</td>
<td>43 57</td>
</tr>
<tr>
<td>Vis. Gust. &amp; Olf.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. dead aphids</td>
<td>9 15</td>
<td>5 6</td>
<td>9 29</td>
<td>14 28</td>
</tr>
<tr>
<td>vs glue</td>
<td>50 24</td>
<td>50 46</td>
<td>25 34</td>
<td>33 27</td>
</tr>
<tr>
<td>B. dead aphids</td>
<td>45 9</td>
<td>6 9</td>
<td>18 13</td>
<td>41 6</td>
</tr>
<tr>
<td>vs honeydew</td>
<td>15 4</td>
<td>0 1</td>
<td>14 8</td>
<td>17 5</td>
</tr>
</tbody>
</table>

* females only, "male" column is M. venablesi and "female" column is E. volucris.
Discussion and Conclusions

In olfactometer experiments, food odours (e.g. mock orange) had the next highest attractive index after the complex that constitutes the "suitable" oviposition site. Syrphids then, can respond to the odour of a flower before (or even without) seeing the associated object or its colour. Olfactory ovipositional stimuli were not so readily recognized at such long ranges however. Only tryptophan and indoleacetaldehyde elicited searching behaviour, and then only in mated females. But even these females displayed only a weak response. Ovipositing insects could not recognize certain individual components of the oviposition stimulus (whole or crushed bean plant and whole aphids) even when green paper covered the mixing zone. And without the visual input by green paper, not even the combined stimuli could be recognized. These limited capacities suggest that a complex interaction of the various components of an oviposition site is involved in the recognition process. In most instances, a single source of information is inadequate, and doubled stimuli are scarcely more helpful. The timing of the prerequisite combination of stimuli, may however, be less critical than their composition - sometimes they may act almost simultaneously; or they may act in sequence as some laboratory tests showed.

Not only do the components of the oviposition site interact in complex ways, but physiological state also affects the behavioural responses. Schneider (1969) noted that, as syrphids age or are deprived of aphids, their ovipositional responses become less selective. And Chandler (1968a) suggested that, as syrphids age, their olfactory ability to select aphids may diminish, thereby unmasking more primitive
host-plant recognition sequences. But in the experiments with *M. venablesi* and *E. volucris*, physiological state of the insect also affected responses to the oviposition plant. Male responses differed in relation to their previous experience with the stimulus. Mated and experienced, but deprived, males were more responsive to the oviposition plant than unmated, experienced males. Among females, those which were mated and also experienced proved to be the most responsive. Next came unmated, experienced individuals, and then mated but naive females. Unmated, naive females were the least responsive.

While various elements of the ovipositional site on glass rods were sufficiently attractive to syrphids to elicit some steps in the oviposition sequence, only aphids could carry that sequence to completion. *M. venablesi* and *E. volucris* thus belong to Chandler's (1968a) truly aphidophagous group, that depends on aphids as the paramount stimulus for oviposition. But even when the end result, oviposition, occurred in these laboratory tests, the insects that laid eggs still probed far longer with their ovipositors for a suitable spot than flies exposed to all available cues in a natural situation. In the laboratory tests, the missing chemical and tactile cues were responsible for the confused responses of the caged flies. A few of the constituent stimuli tested (e.g. the host plant, tryptophan & indoleacetaldehyde) produced somewhat different responses in the olfactometer and on the green glass rods, thereby further strengthening the suggestion that some components of the total stimulus may have different targets at different stages in the behavioural sequence, and therefore can rarely trigger an appropriate behavioural response when they are presented alone.
A hierarchy of behavioural responsiveness can be derived for the stimuli tested by using the frequencies with which stimulus-related activities occurred on the treated rods. The sequence, in decreasing order of attractiveness, is as follows:

1. real aphids
2. honeydew
3. crushed bean
4. tryptophan and indoleacetaldehyde
5. aphid mimicking spots

The possibility that prolonged rearing in the laboratory may influence the responses of gravid syrphids has not been considered in previous work. After trial rearings for several months, and in the light of the report by Chambers (1977, that cited evidence of behavioural changes as well as genetic changes in mass reared insects), I preferred to use only first- and second-generation laboratory-reared insects for behavioural experiments. In particular, my concern was that cage-reared syrphids might, over several generations, be selected for their docility, as they would never need to search for oviposition sites or food, and would usually be kept in relatively crowded conditions. Consequently their response to normal ovipositional cues might differ from wild or nearly wild stock. The experiment in which crushed bean painted on glass rods was exposed to third- and fifth-generation lab-reared *M. venablesi* was the only one in which oviposition took place in the absence of real aphids. Although only one female responded thus, she was a product of prolonged lab-rearing. The fact that the number of lab-reared generations has not been considered in other studies may account for some of the reported discrepancies in behaviour (e.g. Dixon
CHAPTER 3. NEUROPHYSIOLOGY OF SYRPHID ANTENNAL AND OVIPOSITOR SENSILLA
Introduction

All insect chemoreceptors are primary neurons; that is, they lack synapses between their external interface with the environment and the central nervous system. Consequently, selected input to the brain can be directly analysed by recording electrophysiologically from the chemoreceptor. For example, it is possible to make direct recordings of the DC potential in the antennal nerve by means of electroantennograms (EAG). Rather than reflecting the response of individual receptors, however, the EAG reflects the summation of receptor potentials of neurons in the antenna and thus measures the sensitivity of the entire olfactory system. The first such study to use EAGs in this way dealt with the responses of the silk moth, *Bombyx mori*, to olfactory stimuli (Schneider 1957). Since then, EAGs have been extensively used for sex pheromone analysis (e.g. Schneider 1963) and more recently for identifying host plant odours recognized by pest species; e.g. pine oil components used by the wood wasp (Simpson 1976), green plant volatiles used by the Colorado potato beetle (Visser 1979), and grass odours to which locusts respond (Boeckh et al. 1965). Results are most useful when corroborated by behavioural responses to the substances to which receptors are shown to be sensitive; as in the responses of locusts (Kennedy & Moorhouse 1969), and the Colorado potato beetle (Visser & Nielsen 1977) to plant substances.

Recordings from the single receptor have also been used to study differences in specificity between various receptors. Lacher (1967, 1971) recorded responses of thin walled setae on the antennae of *A. aegypti* to several substances found in human body odour, as well as
recording spontaneous base-line activity. Davis (1976) recorded responses to ovipositional attractants in a trichoid sensillum on the antennae of female A. aegypti, while grooved sensilla in females (Davis & Rebert 1972) but not males (Davis 1977), responded to repellents. Other receptors were found to respond to lactic acid (Davis & Sokolove 1976), and even thermal changes (Davis & Sokolove 1975).

The honeydew component of oviposition sites has been shown to be attractive not only to syrphids but also to other aphid predators (Bensaad & Bishop 1976). The green lacewing, C. carnea, after first being attracted to tryptophan in artificial honeydew sprayed on field crops (Hagen et al. 1976), was attracted to this chemical and some of its decomposition products in an olfactometer (van Emden & Hagen 1976). Because green lacewings responded to these olfactory attractants, it is not unreasonable to expect that other aphid predators, including syrphids, may also respond to these components. These chemicals, previously tested in the behavioural experiments just described, were assessed with neurophysiological tests outlined in this chapter.

Visser's (1979) studies on EAG responses of Colorado potato beetles showed that the 6-carbon chain alcohols and aldehydes to which the beetles responded also elicited EAG responses from many other phytophagous insects, including the tobacco horn worm Manduca sexta, a shoot borer Hypsipyle grandella, the ermine moth Yponomeuta sp., the locust Schistocerca gregaria, and the beetle Adoxophyes orana. Visser (op.cit) therefore concluded that this group of compounds was of importance to the host selection process among phytophagous insects. Six-carbon chains have also been shown to be important to the blowfly, C. vicina,
(Kaib 1974) in which one type of antennal receptor for meat odours is especially sensitive to 6-carbon aldehydes, alcohols and ketones. Thus the sensitivity to 6-carbon chain compounds may be even more widespread than Visser (1979) suggested. If insects from diverse families utilize the same types of volatile compounds during host finding, then they may also be used by other nonphytophagous insects besides the blowfly. For example, there are phytophagous as well as aphidophagous syrphids, both of which could use volatile components of green plants to locate their host plants.

Among the insects, contact or gustatory chemoreceptors are found in particularly high numbers on the tarsi, labella and ovipositor. These receptors can be stimulated individually with solutions of chemicals. Labellar hairs have been shown to be sensitive to salts, sugars, and water in *Phormia* (Dethier 1963; Rees 1968) and the labellar hairs of insects that feed at flowers (blowflies, butterflies, and the honeybee) are also sensitive to salts and sugars (Schoonhoven 1968). Maxillary gustatory sensilla of lepidopterous larvae are sensitive to sugars, amino acids, and glycosides. Sensitivity varies with the species and involves both nutritive and non-nutritive plant products (Dethier & Kuch 1971). Such studies again suggest groups of compounds that syrphids may also utilize. Ovipositor gustatory receptors have only recently been recognised (Behan & Ryan 1977; Hooper et al. 1972) and only infrequently studied by single sensillum recordings (Rice 1976). Since some components of the oviposition site are among the compounds previously mentioned, ovipositor-hair recordings should improve our understanding of the process of selection of oviposition sites. In this chapter, I use the EAG technique on female syrphid antennae to test the volatile
substances used in the behavioral studies with the olfactometer and green glass rods (Chap. 2). Contact chemoreceptors on other body parts were tested for their responses to the nonvolatile components of these substances as well.
Materials and Methods

Electroantennograms were obtained in Dr. B.K. Mitchell's laboratory in the Department of Entomology, University of Alberta. Antennae from freshly decapitated laboratory-reared *M. venablesi* females were excised at the first antennal segment. The tip of the third segment was then removed before the base was inserted in the end of a glass micropipette filled with 0.01M NaCl. The pipette was placed over a silver/silver chloride electrode mounted on a Leitz dissecting microscope. The second (recording) electrode, in another micropipette containing 0.01M NaCl, was then placed within the hole in the tip of the third antennal segment.

Signals from the recording electrode passed through a microamplifier and could be viewed on a Tektronix 549 storage oscilloscope or recorded on a Harvard #486, 12-speed chart recorder. Responses were recorded on 35mm Ektachrome slide film, using the oscilloscope storage function, and on photographic paper in a Cine-scope Recorder connected to a second Tektronix oscilloscope. Figure 11 shows the electroantennogramme apparatus.

Solutions to be tested were applied by micropipette until they saturated one half circle of a 1 cm disc of filter paper. The wet filter paper was then placed in a 3 ml disposable Stylex syringe equipped with its needle and 12 cm of plastic tubing having the same bore as the needle. After the desired quantity of air was drawn into the syringe, the tube was sealed to allow the test compound to saturate the air.
Figure 11. Electroantennogramme Apparatus

The electroantennogramme apparatus with dissected antenna (AN), electrical circuitry (electrodes RE and RF), amplifier (MA), air delivery system, delivery tube (DT), syringe (SY), test sample (SF) and stimulus removal funnel (F), all enclosed in a copper mesh cage (CS), with the recording equipment, an oscilloscope (OS), outside.

Legend

AN  antenna  
RE  recording electrode  
RF  reference electrode  
MA  microamplifier  
OS  oscilloscope  
DT  delivery tube  
SY  syringe  
SF  sample on filter paper  
F  funnel  
CS  copper screen  
PA  purified air
An "L" shaped glass tube, tapered at both ends, and with a 5 mm diameter hole at the elbow, was used to deliver the stimulus. A small aquarium air pump was connected by soft rubber tubing to an activated charcoal filter which in turn was connected to the short arm of the L-shaped tube. The tube was positioned in a clamp stand so that the tip of the long side of the "L" was only a few mm from the excised antenna and pointed directly at it. The air forced over the preparation by the pump was carried away by a vacuum line attached to a plastic funnel on the other side of the microscope. The pump provided a constant rate of air flow throughout all experiments. Test substances were introduced into the air stream by inserting the tubing of the syringe into the hole at the elbow of the delivery tube, and injecting the syringe contents quickly and smoothly. All equipment, exclusive of the recorders, was enclosed in a copper mesh cage to eliminate extraneous electrical interference. Room temperature was 22°C.

Preparations of ovipositor and labellar hairs were made by excising whole abdomens and extended mouthparts from freshly killed young M. venablesi or E. volucris females, and mounting them on a micropipette filled with 0.01M NaCl or phosphate buffer. This pipette was then mounted on a silver electrode on a Leitz micromanipulator arm under a Leitz compound microscope (magnification 384X). Individual hairs were located by focusing. Test solutions were placed in another micropipette (bore 4 microns at tip) mounted on the second electrode. The individual hairs were then stimulated by moving the micromanipulator. Responses were viewed on a Tektronix 536 Oscilloscope, sound recorded on a Philips Minilog 4 (0-2500 HZ) tape recorder, and visually recorded on a Honeywell 1858 CRT Visicorder on Kodak direct print Linagraph 2165
paper. As before, all equipment, exclusive of the recorders, was
enclosed in a copper-mesh cage to eliminate extraneous electrical
interference. Figure 12 shows the design of the apparatus for indivi-
dual hair recordings. Room temperature and humidity were controlled at
16°C and 60-70 % relative humidity to prolong the useful life of speci-
mens.
Figure 12. Single Hair Recording Apparatus

This apparatus was used for recording responses of single ovipositor hairs (OP) with the test solutions (TS) in a micropipette. The electrical circuitry and microamplifier (MA) were all enclosed in a copper mesh cage (CS) while the recording equipment, a tape recorder (TR), oscilloscope (OS), and ultraviolet sensitive chart recorder (UV) were outside.

Legend

OP ovipositor pad with chemosensitive hairs
TS test solution
RE recording electrode
RF reference electrode
MA microamplifier
CS copper screen
TR tape recorder with sound production
OS oscilloscope
UV ultra violet sensitive chart recorder
Results

Electroantennogramme Recordings

Two female *M. venablesi* were tested with the following chemicals: green plant volatile substances, trans-2-hexen-1-ol, cis-2-hexen-1-ol, trans-3-hexen-1-ol, cis-3-hexen-1-ol, cis-3-hexenyl-acetate (Roth chemicals), and hexanol-1 (Sigma Chemicals); crushed carnation petals; crushed pea aphids; two other volatile substances of plant origin, methylsalicylate and amyacetate; honeydew and some of its components, tryptophan, indolealdehyde, and indoleacetaldehyde (American Scientific Chemicals).

A positive response or antennal nerve depolarization showed on the oscilloscope as a dip in the baseline followed by a recovery to the original level. Syrphid antennae proved to be excellent preparations for EAGs. Baseline noise was low and the signal was stable, drifting only rarely. Preparations lasted up to 8 h, allowing duplicate testing of many substances.

Controls of dry (dry filter paper disc in the syringe) and moist air (wet disc in the syringe) elicited no response, or only a slight response when an injection was too quick or uneven (Fig. 13 A&B). Dry and moist controls were tested after every 8 or 9 stimulations. All experimental filter paper discs were saturated with the test solution. Variable quantities of test molecules could be administered by varying the volume of saturated air injected. The nerves were allowed one minute to recover between consecutive stimulations since preliminary trials showed that no greater recovery time was required to obtain
maximal response during subsequent stimulations.

Preparations varied slightly in their magnitude of response, but there was rarely any question whether there had been a response. Ten replicates of each of the green plant volatiles elicited positive responses in both antennal preparations. Responses to trans-2-hexen-1-ol and trans-3-hexen-1-ol are shown in Figure 13 C&D. Dose response curves for these plant volatiles were constructed for one antennal preparation (Fig. 15 A & B). The cis-isomers of these two compounds showed no difference in response (Fig. 13D & 14A). The dose response curves for these isomers are shown in Fig. 17 B & C. Positive responses were also obtained for hexanol-1 (Fig. 14B) and cis-3-hexenylacetate (Fig. 14C).

Twenty to thirty pea aphids ground with 2 µl of distilled water produced enough liquid to soak a filter paper disc. Antennae were subjected to 2 ml of saturated air from this syringe. This treatment was repeated twice. Each time a slight reaction was recorded (Fig. 14E). The mean response is plotted in Fig. 17D.

The liquid from crushed white carnation petals was used to soak a filter paper disc. One antenna was given graded tests with this stimulus in amounts of 0.4, 2.0 (Fig. 14D), and 3 ml. It responded with respective deflections of 0.23, 0.46, and 1.24 mv. The response to 2 ml is shown in Figure 17D.

Two other plant volatiles, methylsalicylate and amylacetate, had strong odours easily detectable by humans. These substances were each tested once. There were responses to 2 ml quantities of both chemicals
(Fig. 15 A&B). The magnitudes of the responses are shown in Figure 17D for comparison with other substances tested.

Honeydew was collected by placing a plastic petri dish lid under a heavily infested broad bean leaf overnight. The resulting solids were dissolved in 0.1 ml distilled water and used to saturate a filter paper disc. When 2 ml of saturated air from this syringe were passed over one antenna, there was only a very slight deflection of the baseline (Fig. 15C). Honeydew components, tryptophan, indolealdehyde (both saturated in distilled water) and indoleacetaldehyde (0.01% in distilled water, as used for ovipositor hairs) were applied to antennae 3, 5 and 2 times respectively, with no response (Fig. 15 D&E). Thus, while plant substances and crushed aphids elicited EAG responses from female M. venablesi antennae, honeydew, some of its components, and its oxidation products did not.
Responses to 2 ml quantities of air saturated with each of the following substances:

A) moist air control
B) dry air control
C) trans-2-hexen-1-ol
D) cis-2-hexen-1-ol
E) trans-3-hexen-1-ol

The deflection distance down indicates the quantitative response of the antennal nerve. The form of the dip indicates the smoothness of the application. One second is indicated by two dashes of the line under each response recording. The arrow indicates the point in time of application.
Responses to 2 ml quantities of air saturated with each of the following substances:

A) cis-3-hexen-1-ol
B) hexanol-1
C) cis-3-hexenyl acetate
D) crushed carnation petals
E) crushed aphids

See Figure 13 caption for interpretive details.
Figure 15. Electroantennogramme Responses

Responses to 2 ml quantities of air saturated with each of the following substances:

A) methylsalicylate
B) amylacetate
C) honeydew (in water)
D) indolealdehyde
E) indoleacetaldehyde

See Figure 13 caption for interpretive details.
Figure 16. Green Plant Volatile Substances Dose Response Curves

Dose response curves for three green plant volatiles in mv deflection units against dose of saturated air administered by syringe to a constant air-flow over female *M. venablesi* or *E. volucris* antennae prepared for electroantennogramme responses.

A) trans-2-hexen-1-ol

B) trans-3-hexen-1-ol

C) hexanol-1
Figure 17. Green Plant Volatile Substances Responses and Dose Response Curves

A–C  Dose response curves for three cis-isomers of green plant volatiles in mv deflection units against the dose of saturated air administered by syringe into a constant air flow over female M. venablesi or E. volucris antennae prepared for electroantennogramme responses.

D  EAG responses in mv deflections for 2 ml quantities of air saturated with the following substances:

- aa  amylacetate
- cp  crushed carnation petals
- a  crushed aphids
- ms  methylsalicylate
- h  honeydew
Single Ovipositor Hair Recordings

Ovipositor hairs of both M. venablesi and E. volucris females were stimulated with the following substances: honeydew, tryptophan, indoleacetaldehyde, alanine (as one of the additional amino acids found in honeydew) and sucrose (one sugar found in honeydew). Each substance was used to stimulate numerous contact chemoreceptive hairs on each preparation.

Initially 0.08 M phosphate buffer or 0.05 M NaCl (Fig. 18A) were used as controls, but there was a strong chemoreceptor response to both solutions. Since this reaction appeared to be a response to salt, successively less concentrated NaCl solutions (down to 0.01 millimolar mM) were tested. The response did not weaken proportionally, and remained unchanged even when possible osmolarity effects were eliminated by using 1 mM NaCl in 0.1 M glucose. This response apparently was not to the salt but to the water, since it could be successfully depressed with 10% dimethylsulfoxide (DMSO), which has a molecular structure similar to water and thus could compete for receptor sites. This new procedure effectively blocked the response to water without killing cells, and thus provided the necessary controls. The concentration of 10% DMSO was the lowest that was effective in maintaining control baseline readings between receptor stimulations.

For subsequent tests, therefore, all substances were made up in 1.0 mM NaCl, with and without 10% DMSO. Hairs to be tested were first stimulated with 1.0 mM NaCl, then with 1.0 mM NaCl in 10% DMSO, then with the test solution in NaCl, with and without 10% DMSO. After that sequence was completed they were tested again with 1.0 mM NaCl to ensure
that the DMSO had not adversely affected any cells or their receptor sites.

Four responsive ovipositor preparations from each species were used. As noted, several hairs from each preparation were stimulated by the test substances. Test concentrations used were: 0.01 M indoleacetaldehyde, 1.18% tryptophan (saturated), 0.1 M alanine, and 0.1 M sucrose. Indolealdehyde was not tested. Honeydew was collected with a drop of 1.0 mM NaCl from a glass microscope slide left overnight under a bean leaf heavily infested with pea aphids.

The ovipositor hair responses to honeydew, tryptophan, indoleacetaldehyde, alanine, and sucrose are shown in Figures 18 B,C,D, and 19 A,B respectively. A typical insect chemoreceptor response consists of two stages, phasic and tonic. The initial response (phasic) has closely spaced spikes. This changes to a less frequent firing pattern, the tonic response. Periodicity decreases with continued stimulation, but nerve recovery time is short. Mechanoreceptor responses, which sometimes occurred when the hair was moved during attempts to stimulate it, were far less regular (Fig. 19C). Only qualitative responses were recorded, since only one concentration of each stimulus was used.

Labellar and Tarsal Hair Recordings

There were two labellar preparations for each species. These preparations were tested with 1.0 mM and 10.0 mM NaCl, and 0.01 M indoleacetaldehyde, 0.1 M alanine, 1.18% tryptophan, and 0.1 M sucrose (all in 1.0 mM NaCl). As before, honeydew was collected in 1.0 mM NaCl from a microscope slide placed under a broad bean plant infested with
pea aphids. In one female *E. volucris* that was <1 week old, a few short but otherwise unidentified hairs (Fig. 19D) responded to 0.1 M sucrose in 1.0 mM NaCl. There were no responses or response artifacts to the other substances.

Another young *E. volucris* female was tested for tarsal hair sensitivity to 0.1 M sucrose in 1 mM NaCl. Stimulus artifacts occurred, indicating good contact between hair tip and test solution. No response was recorded on any of several hairs tested. Those particular hairs apparently were not sensitive to sucrose.
Figure 18. Ovipositor Hair Electrical Responses

A) Electrical response of the ovipositor UP contact chemoreceptor to 0.05M NaCl. This is a typical chemoreceptor cell response and in this receptor, shows the response to water by the water sensitive cell.

B) Electrical response of the ovipositor UP chemoreceptor to honeydew.

C) Electrical response of the ovipositor UP chemoreceptor to tryptophan, an amino acid component of aphid honeydew. The response is not so clear as that to water, but was consistently present in this form each time a sensillum was tested.

D) Electrical response of the ovipositor UP chemoreceptor to indoleacetaldehyde, an oxidative product of tryptophan.

The arrow in each tracing indicates the stimulus artifact, or the point at which the test substance makes contact with sensitive cells.
Figure 19. Ovipositor Hair Electrical Responses

A) Electrical response of the ovipositor UP chemoreceptor to the amino acid alanine.

B) Electrical response of the ovipositor UP chemoreceptor to sucrose. Again, the response is not so clear as that to alanine, but was consistent in sensilla repeatedly tested.

C) Electrical response of the ovipositor UP chemoreceptors to mechanical stimulation (with chemical stimulation also). The typical mechanoreceptor response is a series of short bursts of activity.

D) Electrical response of a labellar contact chemoreceptor to sucrose. As in the sucrose response in ovipositor hairs, the form was not typical but remained consistent with repeated stimulations.
Discussion

The discovery of a water receptor on the ovipositor was unexpected, since there is no suggestion in the literature that these syrphids might require water or high humidity for oviposition. Freshly excreted honeydew is wet, however, and a certain amount of water vapour is associated with stomata on the undersides of leaves. In addition, many members of the Milesiinae oviposit in tree rot holes and sap oozes (Maier & Waldbauer 1979a), and *Eristalis tenax* ovipsits in polluted water (Borrer et al. 1976). It seems possible that the syrphid water receptor was evolved for the obvious needs of the Milesiinae but was retained by the aphidophagous forms to confirm the presence of fresh honeydew (and thus live aphids) and/or a favourable aphid habitat on a healthy leaf. Its importance in aphidophagous syrphids is evidenced by the demonstration that at least one of their ovipositor's four chemosensitive neurons remains sensitive to water.

Evans & Mellor (1962) reported a water receptor in long trichoid sensilla on the labellum of *Phormia terranova*, and Rees (1970) studied its mode of action. If water is an important element in oviposition site selection, then one might expect to find an olfactory sensitivity, in addition to the sensitive contact sensilla on the ovipositor. Olfactory water-vapour sensilla have in fact been demonstrated electrophysiologically on the tarsi of the brown dog tick, *Rhipicephalus sanguineus* (Haggart & Davis 1980). Behavioural evidence has suggested the presence of hygrothermic receptors on many other insects (see Dethier 1963) but my results suggest that they are lacking on syrphid antennae. Indeed, water vapour was used as a "moist air control" in EAG
Host-plant selection by phytophagous insects is to a large extent chemosensory. Some plant-feeders are very specific, showing a fine degree of discrimination, whereas others show little specificity (Schoonhoven 1968). The sensory impact of host-plant odours is one of the first and most important triggers setting off the feeding process of a phytophagous insect (op.cit). All of the common green plant volatiles tested here induced a response by the antenna of the aphidophagous *M. venablesi*, just as they have for many kinds of phytophagous insects (Visser 1979). Consequently, for the first time, the present study has demonstrated that the antennae of an aphidophagous insect can also respond to volatile plant substances. The behavioural response, however, was not so clear-cut. Apparently, the odour of a host plant, either whole or crushed, in an olfactometer, by itself will not normally elicit oviposition-site searching behaviour by an aphidophagous syrphid. The taste of crushed bean, on the other hand, was of more interest to ovipositing females than males, inducing them to display at least some activities usually related to searching for oviposition sites.

The absence of an EAG response to tryptophan and indoleacetaldehyde was somewhat surprising, since there was a minor behavioural response during the olfactometer experiments (Chap.2). Although no EAG has been performed on green lacewings, the work of van Emden & Hagen (1976) strongly suggests the presence of antennal olfactory sensilla sensitive to honeydew components. In contrast, the results obtained here suggest that, in syrphids, the responding sensilla are not located on the antennae. The primary function of the ovipositor hairs that did
respond is not olfactory, but gustatory. Nevertheless, some of these hairs might be sensitive enough to respond to air-borne molecules prior to contact. This additional capacity is known to occur in some contact chemoreceptors (Zacharuk 1980) but unfortunately the recording system used here could not demonstrate it. In the present system, the recording electrode was inside the stimulus pipette and thus could record nerve activity only after contact with the hair tip. To detect increased nervous activity prior to contact, the electrode must be inserted through the side wall of the hair. My results therefore cannot resolve the question of olfactory reception by the ovipositor.

Although the respective EAG responses to crushed carnation petals and crushed aphids may have been partly due to flower and aphid odours, they were more probably due to the small concentrations of the same green plant substances which elicited the earlier antennal responses. Both stimuli would be expected to contain these compounds. Volatile flower odours induced searching behaviour in both males and females in the olfactometer trials (Chap.2). Moreover, Kaib (1974) identified three different types of flower odour receptors on the antenna of the blowfly, C. vicina. This evidence suggests that syrphids also have specialized receptors for flower odours, but the EAG response could not be distinguished in my experiments from a possible response to any crushed plant material.

The EAG response to methylsalicylate is probably important because this compound is found in plants, though it is not so common as the green-plant volatiles. Unlike these volatiles, its chemical core structure is cyclic, rather than straight-chained. The only structural ele-
ment it has in common with the volatiles is an exposed hydroxyl group, which may be an important requirement at receptor sites. For example, Visser (1979) changed the position of the terminal hydroxyl group during tests of several short-chain alcohols and aldehydes on the Colorado potato beetle, and found that the EAG response, while still present, was much reduced after the change. If the exposed hydroxyl group is not involved, then the strong odour of methylsalicylate might be readily detected by a more general chemical sense. The form of the response, which was different from that of other chemicals tested (Fig. 15A), provides some evidence for this alternative possibility. When exposed to methylsalicylate, the stimulated nerve took much longer to recover, indicating that this particular molecule continued to stimulate the receptor site longer than the other types of molecules. Weak responses to methylsalicylate have been demonstrated in antennae of the Colorado potato beetle (Visser 1979) and in the blunt-tipped sensilla trichodea, type II, of female *A. aegypti* (Davis 1976).

The contact chemoreceptors on the ovipositor were sensitive to the components of aphid honeydew tested in these trials. Amino acids are known to stimulate some insect gustatory receptors (Dethier & Kuch 1971). Sugars are also commonly detected by contact chemoreceptors (Dethier 1963; Schoonhoven 1968) but these sugar receptors have been identified most often on labella. The labellum of these syrphids also demonstrated a sensitivity to sugar, although the response was not so clear and typical of insect chemosensory responses as were some of the ovipositor recordings. In aphidophagous, as opposed to phytophagous, syrphids, however, sensitivity of the ovipositor to sugars could be most useful during the final stages of locating a suitable place to oviposit.
Final selection of an oviposition site appears to involve the amino acids as well as sugars in honeydew, and perhaps moisture associated with fresh honeydew and/or the leaf surface, in addition to the tactile cues provided by the mechanoreceptors.
DISCUSSION AND SUMMARY
Female aphidophagous syrphids respond to a number of stimuli in the course of their complex oviposition sequence. Some of the first cues are probably visual. Syrphids approach oviposition sites from some distance (Dixon 1959), perhaps responding to the predominant colour of such sites - green - and the form of the vegetation (Chandler 1967). Although the importance of colour has been debated (Bombosch & Volk 1966; Peshken 1965; Dixon 1959; Chandler 1968a), light intensity at least, has been shown to be important, since ovipositing syrphids prefer shaded colonies (Peshken 1965; Sanders 1979). Negative phototaxis, however, comes into play mainly after location of a suitable host plant, whereas vegetation colour, if important, would be one of the first cues utilized.

Behavioural evidence from *E. tenax* (Ilse 1949) and *M. venablesi* (R. Smith pers. comm. 1981) shows that at least these two species have some colour vision. Electrophysiologically, Bishop (1974) demonstrated that *E. tenax* has receptors in its compound eye that are sensitive not only to ultraviolet but also to wavelengths in the visible range. Behavioural evidence from my olfactometer studies suggests that without some appropriate visual stimulus, female syrphids cannot recognize the odours of their oviposition site. Thus, visual colour stimuli are important and must be closely linked with olfactory stimuli in the early stages of oviposition-site recognition.
Oviposition sites also comprise a range of chemical stimuli of which honeydew has been accorded most credit as the source of olfactory stimulation (Schneider 1969; Bombosch & Volk 1966; Dixon 1959). The more recent work of Hagen et al. (1976) and van Emden & Hagen (1976) on green lacewings, however, suggests that the actual attractants are two components of honeydew, tryptophan and indoleacetaldehyde. The absence of an EAG response to honeydew in my experiments with M. venablesi and E. volucris therefore was somewhat surprising, though it was confirmed by the insensitivity of the antenna to both of the components of honeydew tested, tryptophan and indoleacetaldehyde. The EAG findings were contrary to the observed responses, however slight, of mated females in the olfactometer to tryptophan and indoleacetaldehyde. When syrphids were exposed to these chemicals painted on green glass rods, there was a definite behavioural response by females, but not males (Fig. 4). Since the EAG records do not fit the pattern emerging from other data, other olfactory receptors, not on the antenna, must have been responsible for the observed behaviour. As noted earlier, some contact chemoreceptors can also respond to a very strong olfactory stimulus. The contact chemoreceptors on the ovipositor are a possible alternative site, as they displayed sensitivity to both chemicals. Concentrations of tryptophan and indoleacetaldehyde in the olfactometer were higher than those expected to occur naturally, so they may have triggered an olfactory response in these contact chemoreceptors. Such a dual function of contact chemoreceptors has already been shown for the blowfly (Dethier 1972).
Honeydew is not the only component of the oviposition complex, however. In olfactometer trials, the stimulus was not complete without the plant. Most of the green-plant volatiles tested with syrphid antennae were straight chain, 6-carbon saturated and unsaturated alcohols formed by oxidative degradation of plant lipids. They have been reported as volatile components of numerous plant species in various families (Visser and Ave 1978). The Colorado potato beetle, the migratory locust, and the carrot rustfly are among the insects known to be sensitive to 6-carbon alcohols and aldehydes (Guerin and Visser 1980). With evidence that common green-plant volatiles are exploited by other insects, and with behavioural evidence that some syrphids use plant cues almost exclusively for locating ovipositional sites (Chandler 1968a), it is reasonable to expect aphidophagous syrphids to have the capacity to detect green-plant volatiles (see Chap. 3, EAG experiments). That they did not respond to crushed broad bean in the olfactometer (Chap. 2) suggests that some plant odours by themselves may not be a sufficient stimulus to elicit a complete behavioural response in these species.

Chandler (1968a) suggested that, in the syrphids' evolution, the phytophagous habit was the oldest, whereas the entomophagous habit was more recent. The EAG results in this study support his suggestion that the capacity to recognize host plants without aphids is still present in entomophagous species. Chandler (1968a) suggested that this existing response to host plants is normally masked in truly aphidophagous species, but that this masking deteriorates under adverse conditions. The fact that specific responses do diminish with aging, or after long periods when the flies are deprived of contact with hosts (Schneider 1969), is further support for his hypothesis.
In olfactometer observations during this study, neither plants alone nor aphids alone elicited the behavioural response of searching in the two species tested (Chap. 2). It is significant that there was no response to aphids without the plant (and perhaps honeydew). Even though green-plant volatiles are still important components of the stimulus that triggers searching for hosts by entomophagous syrphids, the complete ovipositional response seems to depend on a combination of stimuli. The evidence presented here favours multiple stimulus recognition rather than a masking of the response to a host plant. The EAG response to crushed aphids may very well have been due to the plant components ingested by the aphids. That response certainly was appropriately small when compared with those of the other stimuli (Fig. 14E). Some further support for the suggestion that green-plant volatiles are an important component of the oviposition stimulus comes from the oviposition experiments with the green glass rods. Crushed bean painted on a green glass rod attracted many more syrphids than the control rod, and the activities on this treated rod were related to the stimulus (Fig. 3). Females visited the treated rod significantly more often than males (Fig. 3). Much tasting was involved in these visits, which strongly suggests that the host plant was also contributing gustatory information. This increased attentiveness to the host plant may be another relic of the ancestral phytophagous habit. Since this component does not seem to be completely masked in entomophagous species, it is available to act within the stimulus combination suggested above.
The odour and/or taste of crushed aphids on a glass rod was expected to enhance oviposition, as reported by Dixon (1959). My results with glass rods, however, agreed with results obtained in the olfactometer, that the odour of aphids could not itself induce oviposition in these species. The difference in response to these glass rods and those on which dead aphids were glued was probably in large part due to the visual stimulus of real aphids. Again then, two types of stimulus were linked, and combined stimuli had to be presented before a specific behaviour was released.

Whole aphids on a green glass rod induced oviposition without support from plant stimuli. In these experiments, the plant element of the complex was missing and presumably so were most of the honeydew-related stimuli. Since "normal" oviposition behaviour continued until the ovipositor probed for an appropriate site, olfactory, visual, and perhaps some gustatory cues were sufficiently strong to eliminate the dependence on certain of the plant stimuli. At the stage of ovipositor probing, however, there were behavioural indications that some stimulus was missing. In the syrphids studied, the ovipositor was sensitive to alanine, sucrose, water, honeydew, tryptophan and indoleacetaldehyde (Fig.18). So the ovipositor is not only a stereotactic organ, as evidenced by the presence of a mechanoreceptor in each of the gustatory hairs (Plate 10); it is also capable of recognizing chemical stimuli which identify the oviposition site. These chemical stimuli are related to the presence of honeydew. The glass rod lacked honeydew, though it was probably present on aphids, and they were where some females chose to oviposit. There was also an absence of leaf moisture and fresh honeydew which the water receptor might otherwise have detected.
Perhaps these missing parts of the stimulus are important for ovipositing females that have a wider choice of sites, but when deprivation or limited choice occurs as in the forgoing laboratory tests, the importance of the water stimulus may be diminished along with some of the plant stimuli.

Labellar contact chemoreceptors, had they been as accessible as ovipositor chemoreceptors, would probably also have displayed some of the same sensitivities as the ovipositor hairs. Behavioural evidence for labellar gustatory perception of these chemicals was abundant in the oviposition-rod studies. Both males and females tasted more often on honeydew- and crushed bean-treated glass rods than on controls. Females spent significantly more time tasting and searching on the tryptophan- and indoleacetaldehyde-treated rods than on controls (Fig. 4). In other experiments, when both tryptophan- and indoleacetaldehyde- treated rods were simultaneously presented with honeydew-treated rods, significantly more females than males were attracted to and searched and tasted the tryptophan- and indoleacetaldehyde-treated rods (Fig. 6). Labellar gustation thus seems to be a major step in the behavioural sequence for detecting the ovipositional attractants, tryptophan and indoleacetaldehyde.

In summary, a flow diagram showing possible combinations of stimuli and responses of an ovipositing aphidophagous female is presented to explain the behavioural sequence in oviposition.
Are you hungry? yes → Feed
no →
Do you see the right colour? no → Keep searching
yes →
Do you smell a potential site? plants? honeydew? aphid? no → Keep searching
yes → Hover search
Is there an olfactory gradient? no →
yes →
Is it highest on the dark side of a leaf? yes →
Do you see an aphid pattern? yes → Land and taste with labellum
no →
Do you taste a plant? honeydew? yes → Taste any aphids Are they fresh?
oes no →
Test taste substrate with ovipositor
Are labellum taste cues confirmed? no →
Is honeydew fresh? yes →
Is the plant healthy? yes yes yes → Oviposit
no →
Does the substrate feel right? yes no →
Take off/ preen
LITERATURE CITED


Materials and Methods

Preference Experiments with Natural Flowers

Six varieties of garden flowers grown in a plot protected from insecticides, were presented in cages to lab-reared *M. venablesi* and *E. volucris*. I used the following varieties of flowers: petunia, salpiglossis, phlox, alyssum, zinnia, and 4 o'clocks. One variety at a time was presented in all available colours. During any one trial, either single flowers or clusters of comparable size were presented and the number, sex, and activity of insects at each flower were recorded at five minute intervals. Observation periods ranged from 0.5 to 2 hours, but the majority were 1 hour. Time of day, and the species, age, and the number of insects in the cage were recorded for each observation period. As in experiments with artificial flowers, total observational intervals were combined for each flower type after first comparing species, and time of day differences. Total frequencies of visits by males, females and the sexes combined, to each flower colour were compared with Chi-squared tests. Frequencies of visits devoted to feeding, resting or other activities were also compared in the same statistical tests.

Because numbers of individuals in each cage differed, frequencies among flower types were not directly compared. Instead, an index of attractiveness to compare results from different flowers was used:
<table>
<thead>
<tr>
<th>visits to one flower colour</th>
</tr>
</thead>
<tbody>
<tr>
<td># insects exposed / total time (in hours) of observation</td>
</tr>
</tbody>
</table>
Figure 20. Spectroradiometer Plots of Reflected Light From Artificial Coloured Flowers.

Spectroradiometer values at 10 nm intervals from 200 to 900 nm for light reflected from coloured paper sheets on the floor of a syrphid cage. The colours of paper are A) blue, B) green, C) yellow, and D) red.
Experiments with Artificial Flowers

Four, 10 cm diameter paper flowers (blue, green, yellow, red) each with a sugar cube at its center were placed in a cage of adult syrphids (with 11-13 members of each sex unless otherwise noted). At five-minute intervals, the number of individuals of each sex and their respective activities were recorded for each flower. There were no other food sources in the cage. Spectroradiometer determinations of energy at 10 nm intervals between 200 and 900 nm were taken from light reflected by each colour. Graphs of the spectral compositions are presented in Figure 20. Reflected light was measured from a large sheet of each of the coloured papers placed at a 45° angle to the cage floor, 4-5 cm from the sensor.

First- and second-generation lab-reared *M. venablesi* and *E. volucris* were used in all but one of these experiments *Syrphus opinator*, from the first lab-reared generation were used during one experiment. The tests required 0.5-2.5 h to complete.

At the end of one observation, total number of intervals were summed and added to those for similar experiments on the same species; e.g.

<table>
<thead>
<tr>
<th>species</th>
<th>time</th>
<th># of intervals of observation</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. venablesi</em></td>
<td>AM</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>PM</td>
<td>47</td>
</tr>
<tr>
<td><em>E. volucris</em></td>
<td>AM</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>PM</td>
<td>63</td>
</tr>
<tr>
<td><em>Syrphus</em></td>
<td>AM</td>
<td>18</td>
</tr>
<tr>
<td><em>M. venablesi</em> (males only)</td>
<td>AM</td>
<td>13</td>
</tr>
</tbody>
</table>
To facilitate comparisons, each group with less than 63 intervals was multiplied by a factor that brought it up to 63. Then the frequency of visits to each flower within such groups was also multiplied by this factor.

Chi-squared tests were used to compare spontaneous preferences for flower colour within and between sexes, morning and afternoon visits, and between species.
Real Flower Preference Results

A) Petunias

Six colours of petunia were used; purple, mauve, red, pink, magenta and variegated purple. A single flower of each was placed in a glass vial in the syrphid cage. Thirty male and 32 female M. venablesi (4-5 weeks old) were observed with these flowers for a total of 7 hours; 3.7 in the morning, and 3.3 in the afternoon. These insects had been lab reared for 10 generations. As the number of insects used was the same for each observation period, the mean number of insects per hour was 62.

Proportion of visits spent in various activities were as follows:

<table>
<thead>
<tr>
<th></th>
<th>Feeding</th>
<th>Resting</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>m f both</td>
<td>m f both</td>
<td>m f both</td>
</tr>
<tr>
<td>prop.</td>
<td>0 .29 .26</td>
<td>.92 .44 .49</td>
<td>.08 .27 .25</td>
</tr>
<tr>
<td>freq.</td>
<td>0 36 36</td>
<td>12 56 68</td>
<td>1 34 35</td>
</tr>
</tbody>
</table>

Males spent significantly more visits resting while females showed no differences in amounts of time in each activity.

Morning and afternoon visits differed significantly. A total of 96 visits (88f, 8m) occurred in the morning while only 44 (37f, 7m) occurred in the afternoon. Females visited flowers significantly more
often than males in both morning and afternoon.

In morning observations males preferred the mauve flower (P< 0.01). Females visited all flowers but the purple and red (this was significant at P< 0.001). In afternoon observations, males visited only the purple flower but the numbers of visits were too low to be reliable (7). Females showed a significant preference for the mauve and variegated flowers.

When all visits were combined, males showed no significant preference for any colour while females showed a significant avoidance of purple.

The attractiveness indices for these flowers are:

<table>
<thead>
<tr>
<th></th>
<th>male</th>
<th>female</th>
</tr>
</thead>
<tbody>
<tr>
<td>mauve</td>
<td>0.20</td>
<td>1.09</td>
</tr>
<tr>
<td>purple</td>
<td>0.23</td>
<td>0.03</td>
</tr>
<tr>
<td>pink</td>
<td>0.03</td>
<td>0.94</td>
</tr>
<tr>
<td>red</td>
<td>0.03</td>
<td>0.13</td>
</tr>
<tr>
<td>magenta</td>
<td>0.00</td>
<td>0.44</td>
</tr>
<tr>
<td>variegated</td>
<td>0.00</td>
<td>1.28</td>
</tr>
</tbody>
</table>

The order of preference for males is that listed, but females preferred in order; variegated purple, mauve, pink, magenta, red then purple.
Phlox

Five colours of phlox were presented together in syrphid cages; peach magenta, purple, pink (with a dark center), and white. Small clusters of flowers were used and clusters were the same size. Cages with between 38 and 40 *E. volucris* of each sex were observed for 3.5 afternoon hours while *M. venablesi* (31 males, 30 females) were observed for 2 afternoon hours. Morning observations consisted of 1 *E. volucris* hour, and 3 hours of females only, both species (25 M., 8 E.) for a total of 9.5 hours. All insects were first and second generation lab-reared and up to one week in age. Proportions and frequencies of visits spent in various activities were as follows:

<table>
<thead>
<tr>
<th></th>
<th>Feeding</th>
<th>Resting</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>m</td>
<td>f</td>
<td>both</td>
</tr>
<tr>
<td>prop.</td>
<td>.05</td>
<td>.17</td>
<td>.13</td>
</tr>
<tr>
<td>freq.</td>
<td>6</td>
<td>36</td>
<td>42</td>
</tr>
</tbody>
</table>

(totals for males and females and both were used for respective proportions)

Both males and females spent significantly more visits (*P* < .001) resting than in any other activity. These flowers may not have had attractive nectar or pollen sources for syrphids. The lower number of male visits reflected in part, the three hours of female only observation.
Proportions and frequency of visits to each flower were as follows:

<table>
<thead>
<tr>
<th></th>
<th>magenta</th>
<th>peach</th>
<th>purple</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>m</td>
<td>f</td>
<td>b</td>
</tr>
<tr>
<td>freq.</td>
<td>40</td>
<td>47</td>
<td>87</td>
</tr>
<tr>
<td>prop.</td>
<td>.34</td>
<td>.22</td>
<td>.26</td>
</tr>
</tbody>
</table>

* white was used in fewer experiments so was not compared in Chi squared tests.

Only males showed a slight preference for one colour (magenta) and only in morning visits (P< 0.01). Female visits and afternoon male visits were as expected by chance. The overall number of visits to each flower showed there were no significant preferences.

When species were considered separately for their respective spontaneous preferences, \textit{E. volucris} males showed a significant (P< 0.001) difference from the expected. They visited pink and magenta flowers most often while showing least interest in the purple flower.

The attractiveness indices for these flowers were:

<table>
<thead>
<tr>
<th></th>
<th>male</th>
<th>female</th>
</tr>
</thead>
<tbody>
<tr>
<td>magenta</td>
<td>1.38</td>
<td>1.38</td>
</tr>
<tr>
<td>peach</td>
<td>0.73</td>
<td>2.01</td>
</tr>
</tbody>
</table>
Salpiglossis

Three afternoon hours (2 Meta. 1 Eup.) were spent observing 12 males and 8-12 females, first generation lab-reared, 2-3 week old adults. Six different coloured single salpiglossis flowers were introduced simultaneously. Salpiglossis can be highly variegated with yellow or plainer in colour to the human eye. Three of each type were presented: variegated mauve with yellow, burgundy with yellow, light purple with yellow, plain red, pink, and purple. Mean numbers of insects per observed hour was 22.6.

Proportions and frequencies of visits spent in various activities were as follows:

<table>
<thead>
<tr>
<th></th>
<th>Feeding</th>
<th>Resting</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>m f both</td>
<td>m f both</td>
<td>m f both</td>
</tr>
<tr>
<td>prop.</td>
<td>.10 .05 .07</td>
<td>.80 .74 .77</td>
<td>.10 .21 .16</td>
</tr>
<tr>
<td>freq.</td>
<td>3 2 5</td>
<td>24 29 53</td>
<td>3 8 11</td>
</tr>
</tbody>
</table>

As with Phlox, significantly more visits (P< 0.001) were concerned with resting rather than feeding.
Frequency and proportion of visits to each flower colour were as follows:

<table>
<thead>
<tr>
<th></th>
<th>mauve/ yellow</th>
<th>burgundy/ yellow</th>
<th>lt.purple/ yellow</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>m</td>
<td>f</td>
<td>b</td>
</tr>
<tr>
<td>prop.</td>
<td>.05</td>
<td>.17</td>
<td>.11</td>
</tr>
<tr>
<td>freq.</td>
<td>3</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>red</td>
<td>pink</td>
<td>purple</td>
</tr>
<tr>
<td>prop.</td>
<td>.52</td>
<td>.04</td>
<td>.29</td>
</tr>
<tr>
<td>freq.</td>
<td>30</td>
<td>2</td>
<td>32</td>
</tr>
</tbody>
</table>

In *M. venablesi* visits, males spent a significantly greater (P < 0.001) number of visits on the red flower with relatively few visits to other colours. Females showed a slighter preference for burgundy/yellow (P < 0.01). *E. volucris* males and females showed the same preferences; males for the red flower and females for the burgundy/yellow (P < 0.001). These sex differences remained when species visits were combined.

The attractiveness indices for these flowers were:

<table>
<thead>
<tr>
<th></th>
<th>males</th>
<th>females</th>
</tr>
</thead>
<tbody>
<tr>
<td>mauve/yellow</td>
<td>0.38</td>
<td>1.35</td>
</tr>
<tr>
<td>burgundy/yellow</td>
<td>0.25</td>
<td>3.90</td>
</tr>
<tr>
<td>lt.purple/yellow</td>
<td>0.50</td>
<td>1.00</td>
</tr>
<tr>
<td>red</td>
<td>3.75</td>
<td>0.30</td>
</tr>
<tr>
<td>pink</td>
<td>1.88</td>
<td>0.15</td>
</tr>
<tr>
<td>purple</td>
<td>0.50</td>
<td>1.05</td>
</tr>
</tbody>
</table>
Females seemed to be more attracted to the variegated flowers while males seemed attracted more to the plainer coloured flowers.

Alyssum

Four colours of alyssum; yellow, white, purple and pink were presented in equally sized clusters to cages of syrphids. One experimental cage had both sexes of both species (Meta. 9m 12f, Eup. 15m 18f). A 1.5 hour morning and 3 hours of afternoon observation were done with this cage. Other morning experiments totaled 3 hours (Meta.=2, Eup.=1). All insects were first or second generation lab-reared and up to 2 weeks in age. Cages (except for the mixed species cage) had means of 33 males (+ or - 8.7) and 36 females (+ or - 9.6) in them.

Frequency and proportion of visits spent in various activities was as follows:

<table>
<thead>
<tr>
<th></th>
<th>Feeding</th>
<th>Resting</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>m</td>
<td>f</td>
<td>both</td>
</tr>
<tr>
<td>prop.</td>
<td>.84</td>
<td>.96</td>
<td>.92</td>
</tr>
<tr>
<td>freq.</td>
<td>427</td>
<td>892</td>
<td>1319</td>
</tr>
</tbody>
</table>

The frequency of feeding visits was significantly greater in both sexes (P< 0.001) when tested by the Chi squared statistic. This differed from the other flowers, where visits were more frequently not for feeding. Alyssum must be an attractive nectar and/or pollen source for both sexes. Frequency and proportion of visits to each flower colour were as follows:
<table>
<thead>
<tr>
<th></th>
<th>yellow</th>
<th>white</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>m</td>
<td>f</td>
</tr>
<tr>
<td>prop.</td>
<td>.39</td>
<td>.64</td>
</tr>
<tr>
<td>freq.</td>
<td>200</td>
<td>590</td>
</tr>
</tbody>
</table>

|       | m      | f     | b     |
| prop. | .14    | .07   | .10   |
| freq. | 70     | 67    | 137   |

Overall visits by males were compared with the Chi squared statistic and found to significantly (P< 0.001) favour yellow, as did the females. White was the colour of second choice for both, followed by pink and purple.

In morning experiments, both sexes of both species showed a significant (P< 0.001) preference for yellow except for E. volucris males. In afternoon experiments the same pattern existed with significantly more visits to the yellow flower except for E. volucris males. These males preferred showed no preference in the morning while favouring the purple flower in the afternoon (P< 0.001).

Females had a significantly greater frequency of feeding visits than males while males rested significantly more often than females (P< 0.001 for both). Neither sex participated in "other" activities to a greater degree than the other.
The attractiveness indices for each flower colour for each sex were:

<table>
<thead>
<tr>
<th></th>
<th>male</th>
<th>female</th>
</tr>
</thead>
<tbody>
<tr>
<td>yellow</td>
<td>7.59</td>
<td>20.11</td>
</tr>
<tr>
<td>white</td>
<td>4.25</td>
<td>6.41</td>
</tr>
<tr>
<td>pink</td>
<td>4.86</td>
<td>2.76</td>
</tr>
<tr>
<td>purple</td>
<td>2.66</td>
<td>2.28</td>
</tr>
</tbody>
</table>

Both sexes had the same order of attraction to flower colours. The higher indices of these flowers compared to the others is also reflected in the relatively high proportion of visits to number of insects exposed. These flowers were clearly the most attractive of all tested.

Zinnias

Five colours of zinnia; light and dark orange, light and dark pink, and green, were presented in cages of E. volucris or M. venablesi with a mean of 34 males (+ or - 9.7) and 39 females (+ or - 12.6). Three morning hours (Meta.= 2, Eup.= 1) and two afternoon hours (Meta.= 1, Eup.= 1) were observed. All insects were second generation lab-reared and up to one week of age.

Proportion and frequency of visits spent in various activities were as follows:

<table>
<thead>
<tr>
<th>Feeding</th>
<th>Resting</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(walk, hover, preen)</td>
</tr>
<tr>
<td>m</td>
<td>f</td>
<td>both</td>
</tr>
<tr>
<td>m</td>
<td>f</td>
<td>both</td>
</tr>
<tr>
<td>m</td>
<td>f</td>
<td>both</td>
</tr>
<tr>
<td>m</td>
<td>f</td>
<td>both</td>
</tr>
</tbody>
</table>


Both males and females spent significantly more (P<0.001) visits resting than in other activities. When compared to males though, females spent a greater proportion of their visits feeding than males.

Proportions and actual frequencies of visits to each colour of flower were as follows:

<table>
<thead>
<tr>
<th>Colour</th>
<th>Light</th>
<th>Dark</th>
<th>Light</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>orange</td>
<td>orange</td>
<td>pink</td>
</tr>
<tr>
<td>m</td>
<td>.14</td>
<td>.16</td>
<td>.14</td>
</tr>
<tr>
<td>f</td>
<td>.16</td>
<td>.15</td>
<td>.22</td>
</tr>
<tr>
<td>b</td>
<td>.15</td>
<td>.14</td>
<td>.19</td>
</tr>
<tr>
<td>freq.</td>
<td>14</td>
<td>19</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>26</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>33</td>
<td>40</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>23</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>23</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>23</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>43</td>
<td>43</td>
</tr>
</tbody>
</table>

There were no significant differences in colour preference for overall results of males or females or results combined. When morning and afternoon results were separated, morning showed no preferences while in the afternoon there was a slight avoidance of the dark orange flower (P<0.01).
Attractiveness indices for these flowers for each sex were as follows:

<table>
<thead>
<tr>
<th></th>
<th>males</th>
<th>females</th>
</tr>
</thead>
<tbody>
<tr>
<td>dark orange</td>
<td>0.41</td>
<td>0.48</td>
</tr>
<tr>
<td>light orange</td>
<td>0.41</td>
<td>0.66</td>
</tr>
<tr>
<td>dark pink</td>
<td>0.67</td>
<td>0.48</td>
</tr>
<tr>
<td>light pink</td>
<td>0.93</td>
<td>0.64</td>
</tr>
<tr>
<td>green</td>
<td>0.44</td>
<td>0.71</td>
</tr>
</tbody>
</table>

As was expected, all indices were low in value and similar in both sexes.

4 O'clocks

Only one hour of observation with second generation *M. venablesi* up to one week in age with four flower colours was recorded; reddish purple, variegated pink with white, white, and yellow. The experiment was done in the morning with 32 males and 31 females.

Proportion of visits spent in various activities was as follows:

<table>
<thead>
<tr>
<th></th>
<th>Feeding</th>
<th>Resting</th>
<th>Others (walk, hover, preen)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>m</td>
<td>f</td>
<td>both</td>
</tr>
<tr>
<td>prop.</td>
<td>.40</td>
<td>.92</td>
<td>.83</td>
</tr>
<tr>
<td>freq.</td>
<td>4</td>
<td>45</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>m</td>
<td>f</td>
<td>both</td>
</tr>
<tr>
<td>prop.</td>
<td>.20</td>
<td>.02</td>
<td>.05</td>
</tr>
<tr>
<td>freq.</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>m</td>
<td>f</td>
<td>both</td>
</tr>
<tr>
<td>prop.</td>
<td>.40</td>
<td>.06</td>
<td>.12</td>
</tr>
<tr>
<td>freq.</td>
<td>4</td>
<td>3</td>
<td>7</td>
</tr>
</tbody>
</table>
Males showed no preference for any activity, while females fed significantly more often (P< 0.001), almost to the exclusion of other activities.

Frequency and proportion of visits to each flower colour were as follows:

<table>
<thead>
<tr>
<th></th>
<th>red/purple</th>
<th>var. pink/white</th>
</tr>
</thead>
<tbody>
<tr>
<td>prop.</td>
<td>m f b</td>
<td>m f b</td>
</tr>
<tr>
<td></td>
<td>0 0.10 0.08</td>
<td>0.90 0.57 0.63</td>
</tr>
<tr>
<td>freq.</td>
<td>0 5 5</td>
<td>9 28 37</td>
</tr>
</tbody>
</table>

Chi squared analysis of the frequency of visits to each colour showed significantly more (P< 0.001) male and female visits to the variegated pink/white flower (though male frequencies were too low to be reliable). The yellow flower received the second greatest proportion of visits by both sexes.

The attractiveness indices for these flowers were:

<table>
<thead>
<tr>
<th></th>
<th>male</th>
<th>female</th>
</tr>
</thead>
<tbody>
<tr>
<td>red/purple</td>
<td>0.00</td>
<td>0.16</td>
</tr>
<tr>
<td>var. pink/white</td>
<td>0.28</td>
<td>0.90</td>
</tr>
<tr>
<td>yellow</td>
<td>0.03</td>
<td>0.42</td>
</tr>
<tr>
<td>white</td>
<td>0.00</td>
<td>0.10</td>
</tr>
</tbody>
</table>
The indices reflect the significant preference recorded for the variegated pink and white flower, followed by the yellow flower as second choice. Unfortunately only one hour was observed with this flower as at least for females, it seemed to be an attractive feeding source similar to alyssum.

Only two of the flowers presented to insects represented favourable food sources, alyssum and 4 o'clocks. In these, preferences by males and females were for the same flower colour but the same colour was not preferred in each type of flower.

For the other flowers, visits were mainly concerned with resting, and each variety showed different species preferences. There was no overall spontaneous attraction for any one common colour, at least as discerned by the human eye. Most flowers used in experiments were placed either under UV light or photographed with a UV filter. Their various fluorescences or lack of it was noted.

Of the petunias, only the pink showed enough UV reflectance to distinguish the flowers from the foliage. Alyssum flowers all appeared flat and dark like the foliage when photographed in the garden. When examined in a UV chamber however, the white flower petals reflected slightly, contrasting with a darker center. Of the zinnias placed in the UV chamber, (green, pink and dark orange) only exposed pollen fluoresced and was quite apparent against the flat dark background of the flowers. Only the pink phlox with the red center was examined under UV light. It showed none of the contrast apparent under white light, it appeared flat and black.
The presence of reflected UV light then seemed to have little bearing on the colour choices where noted.

**Paper Flower Experiments**

The number of visits per flower (adjusted figures, see materials and methods) for each category of experiment are given below:

<table>
<thead>
<tr>
<th>species</th>
<th>time</th>
<th>m</th>
<th>f</th>
<th>b</th>
<th>m</th>
<th>f</th>
<th>b</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. venablesi</em></td>
<td>AM</td>
<td>68</td>
<td>65</td>
<td>133</td>
<td>61</td>
<td>89</td>
<td>149</td>
</tr>
<tr>
<td></td>
<td>PM</td>
<td>56</td>
<td>52</td>
<td>108</td>
<td>60</td>
<td>62</td>
<td>122</td>
</tr>
<tr>
<td><em>E. volucris</em></td>
<td>AM</td>
<td>32</td>
<td>10</td>
<td>42</td>
<td>35</td>
<td>3</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>PM</td>
<td>38</td>
<td>20</td>
<td>52</td>
<td>19</td>
<td>19</td>
<td>38</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>species</th>
<th>time</th>
<th>m</th>
<th>f</th>
<th>b</th>
<th>m</th>
<th>f</th>
<th>b</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. venablesi</em></td>
<td>AM</td>
<td>61</td>
<td>68</td>
<td>128</td>
<td>72</td>
<td>33</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>PM</td>
<td>47</td>
<td>66</td>
<td>113</td>
<td>62</td>
<td>49</td>
<td>111</td>
</tr>
<tr>
<td><em>E. volucris</em></td>
<td>AM</td>
<td>52</td>
<td>4</td>
<td>56</td>
<td>18</td>
<td>39</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>PM</td>
<td>42</td>
<td>6</td>
<td>48</td>
<td>29</td>
<td>26</td>
<td>55</td>
</tr>
</tbody>
</table>

*M. venablesi* and *E. volucris* were compared extensively as most experiments involved these two species. *M. venablesi* females showed a spontaneous preference for the blue flower ($P < 0.001$) in the morning experiments while the yellow flower received the fewest visits. There was no preference in afternoon experiments. Males showed no preference at any time. *E. volucris* females showed a preference in the morning for
the yellow flower (P<0.001), while males showed a preference for the green flower (P<0.001). Females devoted more visits to the yellow flower in the afternoon but not at the P=0.001 level. There was a significant rejection of the green flower for feeding (by females).

There was no significant difference between number of visits in morning and afternoon for each species. When individual colours were compared between morning and afternoon, *M. venablesi* visits showed no difference in preference, not even in female visits to blue. When morning and afternoon *M. venablesi* visits are combined, the female preference for blue is still significant (P<0.001). When visits to individual colours are compared between morning and afternoon for *E. volucris* only, females spent significantly less time visiting the blue flower in the morning over the afternoon. Combined morning and afternoon *E. volucris* visits still showed a preference for green by males and for yellow by females. Females also visited green less often than any of the other colours.

A comparison of total visits by each species shows that *M. venablesi* made a significantly greater number of visits than did *E. volucris*. The same held true for number of visits to each colour.

*Syrphus opinator* experiments were paired with an identical experiment with *E. volucris* so that numbers of observation intervals were similar (18 *Syrphus*, 21 *Eupeopdes*) and individuals in the respective cages were almost equal (40m 30f *Syrphus*, 39m 41f *Eupeodes*). All *Syrphus* results were multiplied by a factor of 1.17 to compare them with *Eupeodes*. *Eupeodes* experiments were all afternoon while *Syrphus* were all morning but as this had caused no differences in past experiments,
they were considered equivalent. Resultant visits to each flower colour by each sex of each species were as follows:

<table>
<thead>
<tr>
<th>species</th>
<th>Red m</th>
<th>Red f</th>
<th>Blue m</th>
<th>Blue f</th>
<th>Green m</th>
<th>Green f</th>
<th>Yellow m</th>
<th>Yellow f</th>
<th>Both m</th>
<th>Both f</th>
<th>Both m</th>
<th>Both f</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syrphus</td>
<td>21</td>
<td>0</td>
<td>21</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>9</td>
<td>0</td>
<td>9</td>
<td>8</td>
<td>11</td>
<td>19</td>
</tr>
<tr>
<td>Eupeodes</td>
<td>6</td>
<td>10</td>
<td>16</td>
<td>6</td>
<td>7</td>
<td>13</td>
<td>20</td>
<td>0</td>
<td>20</td>
<td>9</td>
<td>5</td>
<td>14</td>
</tr>
</tbody>
</table>

S. opinator males showed a significant preference for red with a rejection of blue by Chi squared analysis. S. opinator females showed a significant preference for yellow. In fact, they fed almost exclusively on the yellow flower. E. volucris males, as expected from the earlier experiments, significantly preferred (P < 0.01) the green flower. Females did not feed at all on the green and they avoided it in previous afternoon experiments.
Discussion

Real Flower Spontaneous Colour Preferences

There was nothing of consistent attraction between flower species either in visible reflected light or UV reflected light. The attraction of the yellow alyssum may have had more to do with "visible" light or the insects' special sensitivity to yellow light. With the 4 o'clocks, the patterned pink and white flower was more attractive than the yellow. This may have had to do with an attraction to patterns. Further evidence came from the salpiglossis observation that females found the variegated patterned flowers most attractive (while males found the reverse) even though there was no appreciable amount of feeding on them. The other variegated flower preferred by females was the variegated purple petunia. The yellow 4 o'clocks did not appear as bright to the human eye as the yellow alyssum, and this may have also been the case for syrphids.

That syrphids can probably distinguish wavelengths as long as yellow has been shown by classical training experiments for *Eristalis tenax* by Ilse (1949) and for *M. venablesii* by Smith (1981 pers. comm.). In the latter experiments, lab reared male and female *M. venablesii* were trained to yellow and blue but were not able to recognize (i.e. train to) red food sources.

This suggests they are sensitive to wavelengths up to approximately 590 nm, but not to those above. Bishop (1974) has shown with retinal cell recordings, that *E. tenax* also has functional UV receptors though these have not been shown to operate at the behavioural level yet. More
recently, other photoreceptive cells have been found in the compound eye of *E. tenax*. Besides a UV receptor with peak sensitivity at 340-360 nm, Horridge et al (1975) showed the existence of a UV and blue sensitive cell with peaks at 350 and 450, and a "visible" light receptor cell with a large peak at 520 nm (green) and smaller peaks up into the yellow range.

It seems plausible that other syrphids, especially those feeding in the same habitats as *E. tenax* (i.e. flowers) possess the same visual sensitivity. The spontaneous preferences for paper flowers and real flowers shown here are evidence for colour discrimination. Some other diptera tested for spontaneous responses to colour and showing no preferences, are *Lucilia* spp. (Tachinidae), *Calliphora* spp. (Calliphoridae) and *Sarcophaga* spp. (Sarcophagidae) (Kugler 1956).

**Spontaneous Colour Feeding Preferences: Artificial Flowers**

Outdoor observations suggested that there might be differences in feeding preferences between morning and afternoon. Although there was no difference in total visits between the two periods, the three preferences that were displayed all appeared during the morning experiments. In fact, most outdoor feeding was observed during the morning hours. It therefore seemed possible that the overall greater number of visits by *M. venablesi* and the females preference for blue might be associated with their outdoor feeding habits. *M. venablesi* required for indoor colonies could be caught most often near or on flowers, whereas *E. volucris* was found mainly along grassy verges i.e. where uncut grass had gone to flower. Perhaps this attachment to grassy edges may help to
explain the preference for green that *E. volucris* males exhibited, and may also explain observed interspecific differences in female preferences.

The actual colours that species preferred may be more apparent than real. The four colours were chosen by human colour perception. Even the information from the spectroradiometer is only of limited value in making such selections. This instrument gives qualitative information about spectral composition, not about an insects' perceptions of that composition. It may be more important to note that each coloured paper reflected the whole spectrum of wavelengths, though in differing amounts, that were related to the colour. Since all wavelengths were present in differing amounts, the insect eye need not have been responding to the one colour the human eye perceived in any one paper flower.

Intensity varied between flowers, as did contrast with the white cage floor. Brightness also was a factor that could not be easily controlled, since it is a subjective quality dependent on the sensitivity of a particular species to a particular stimulus. The subjective nature of brightness has not often been defined in insect colour vision experiments (*Neumeyer 1980*), for it depends on the differing sensitivity of a particular insect eye to various wavelengths. Sensitivity in turn depends on how many elements or cells in the eye are sensitive to a particular quality of light. For instance, if the eye is very sensitive to yellow, the amount of yellow in any paper flower will contribute to its apparent brightness. Electroretinogrammes offer a more objective method of determining an individuals' perception of brightness.
Nevertheless, in these experiments with coloured papers, even though brightness was not known, nor intensity controlled, it is worth noting the fact that different species had different spontaneous preferences. If brightness or intensity were the only factors involved, all species and both sexes should have displayed a narrow range of responses. That they didn't suggests that hue was also important.