FEEDING BEHAVIOUR OF *MARUCA TESTULALIS* (GEYER) (LEPIDOPTERA : PYRALIDAE) IN RELATION TO ITS HOST AND NON-HOST PLANTS

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THESIS SUBMITTED TO THE FACULTY OF SCIENCE, RIVERS STATE UNIVERSITY OF SCIENCE AND TECHNOLOGY, PORT HARCOURT, NIGERIA, IN PARTIAL FULFILLMENT FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D) (ENTOMOLOGY)

JUNE, 1991

DEDICATION

I dedicate this work to my parents, Mr Aloysius Mudebo Wamaniala and Madam Fidelis Kwaga Wamaniala without whose good upbringing, guidance and encouragement I wouldn't have attained this goal.

DECLARATION

The contents of this thesis are the product of my original work and have not been presented for a degree in any University.

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The work reported in this thesis has been submitted with our approval as supervisors.

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ABBREVIATIONS USED IN THE TEXT

°C	Degrees Centigrade
ADRB	Average duration of rest bout
Anova	Analysis of variance
ARPPIS	African Regional Postgraduate Programme in Insect Science
AVDFB	Average duration of a feed bout
AVDMB	Average duration of a movement bout
C.P.R.P	Crop Pest Research Programme
cc	Cubic centilitre
Conc.	Concentration
DMRT	Duncan's Multiple Range Test
g	gram
GLM	General linear Model
Hr	Hour
I.C.I.P.E	International Centre of Insect Physiology and Ecology
I.I.T.A	International Institute of Tropical Agriculture
Lat.	Lateral styloconica sensilla
LD	Light Darkness regime
M	Molar
M.P.F.S	Mbita Point Field Station
Med.	Medial styloconica sensilla
mg	milligram
mm	millimetre

mM	millimolar
mm^2	Square millimetre
mV	Millivolts
NFB	Number of feeding bouts
NMB	Number of movement bouts
NRB	Number of rest bouts
P	Maxillary palp
Rep.	Replicate
S.E	Standard error
S.P.R.U	Sensory Physiology Research Unit
SAS	Statistical Analysis System
TDF	Total duration of feeding
TDM	Total duration of movement
TDR	Total duration of rest

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The host plant pods and flowers evoked higher feeding responses than the leaves among the fourth instar larvae in choice as well as no choice tests. First and fourth instar larvae did not feed on leaves of common bean because they were trapped by the dense trichome mat on both surfaces of the leaf and eventually died. This physical defence mechanism may be potentially useful in the management of *M. testulalis*. Fourth instar larvae unlike first instar did not feed at all on cotton leaves and flowers. There were no obvious reasons for this but physical as well as chemical factors may be playing an important role in bringing about the observed responses.

Pure compounds tested using agar cellulose gel and cellulose acetate paper discs included, sugars (sucrose, fructose and glucose), amino acids (methionine, glutamine and glycine), and alkaloids (nicotine and tomatine). All the sugars were effective phagostimulants. Dose response curves relating to feeding bioassays indicated that 0.1- 0.2 M. are the optimum concentration range above which responses to the sugars declined. Methionine and glutamine also stimulated feeding but the response to glycine was very low. Nicotine and tomatine were deterrents. Aqueous extracts from pods and flowers of cowpea and common bean elicited much better feeding responses

than the leaves extracts in the fifth instar larvae. Aqueous extracts from cotton leaves and flowers elicited much lower feeding responses than the host plant extracts. This reduced feeding on cotton extracts would reflect absence of chemical feeding stimulants and/or presence of non-polar deterrents.

Electrophysiological bioassays showed that aqueous extracts from host and non-host plant parts elicited action potentials in the gustatory neurones of the lateral and medial sensilla styloconica on the maxillary galea as well as the sensilla on the maxillary palp.

The palpal sensilla were sensitive to sucrose, NaCl, methionine, glutamine and aqueous extracts of host and non-host plants. Aqueous extracts from different host plant parts evoked higher impulse frequencies than the corresponding extracts from the non-host plant. Furthermore, the spike frequency evoked by a given extract appeared to relate well to results of the feeding bioassays. It appears that inputs into the CNS from the palpal tip sensilla complement those from the styloconica sensilla in order to bring about the observed feeding responses.

ACKNOWLEDGEMENTS

I wish to thank most sincerely Dr Samuel. M. Waladde my principal supervisor and senior research scientist at ICIPE for the deep interest he took in this work and for the immense guidance he rendered in the supervision, discussion and critical evaluation of this thesis.

I also wish to record my gratitude to Professor K.N. Saxena my second supervisor who advised and assisted in the planning of the experiments. The assistance given by my University Supervisor Prof. R. Kumar as well as Dr Okwakpam of the Dept of Biological Sciences Rivers State University of Science and Technology, Port Harcourt Nigeria, is gratefully acknowledged.

Throughout my research period Professor Z. T. Dabrowski the ARPPIS academic coordinator kindly and efficiently catered for every need and was always ready to attend to my diverse problems.

I am also very indebted to Dr P. Njagi of the Sensory Physiology Research Unit who gave me a lot of inspiration in the course of this work and spared every moment to train me in the use of

electrophysiological equipment. The tremendous technical assistance offered by Mr H. Kahoro and Mr S. Ochieng of the S.P.R.U and Messrs Shem. Otieno, John Obara and P. Odawa of C.P.R.P, M.P.F.S. will be hard to forget.

My gratitude also goes to Mr D. Munyinyi and J. Ngoya of the biomathematics section for their valuable assistance in the statistical analysis of my data and for willingly instructing me in the use of computer softwares of all sorts.

I am also grateful to all my colleagues in ARPPIS for their support during the research and discussions which helped to shape this thesis.

Finally, I am very grateful to Director of ICIPE and Chairman of the ARPPIS Academic Board Professor T.R Odhiambo for having considered me worth of the scholarship which was kindly granted by the Germany academic exchange service (D.A.A.D.).

CHAPTER 1

GENERAL INTRODUCTION

The dietary legumes, cowpea [*Vigna unguiculata* (Walp)] and common beans [*Phaseolus vulgaris* (L.)] provide a major source of high quality protein as well as vitamin B to most subsistence farmers in Africa and other parts of the world (Anon, 1981). However, grain yield of both legumes, particularly cowpea, is seriously hampered by the lepidopteran pod borer, *Maruca testulalis* Geyer (Lepidoptera:Pyralidae) which is one of the pest complex of cowpea (Plate 1). It is capable of infesting and destroying virtually all reproductive parts of the plant (Taylor, 1978). Damage to the leaves , flowers and developing seeds constitutes a major limitation to cowpea production. Other pests attacking cowpea belong to the orders: Coleoptera, Hemiptera, Thysanoptera and Diptera (Jackai and Daoust, 1986).

M. testulalis is distinguished by the colour pattern of its wings which are brown with white spots. The larva has a whitish to yellowish white background colour bearing dark spots on each segment. On the dorsal surface these spots form 2 longitudinal rows

Plate 1: A fifth instar larva of cowpea pod borer, *Maruca testulalis* (x8).

characteristic of *M. testulalis* larvae (Schmutterer, 1968). When it is about to pupate it changes its colour to green. The life cycle of *M. testulalis* has been studied by several workers (Taylor, 1967, Koehler and Mehta, 1972; Akinfenwa, 1975; Odebiyi, 1981). Larval development has been variously reported to last 8-17 days (Taylor, 1967), 10-14 days (Akinfenwa, 1975) or 16-17 days (Odebiyi, 1981). In our laboratory it lasts 16-18 days. These variations usually depend on the nature of diet and temperature. There are 5 larval instars followed by a pupal instar lasting 6-9 days (Taylor, 1967).

M. testulalis larvae are oligophagous; they feed on various cultivated as well as wild leguminous plants (Taylor, 1967). In northern and southern Nigeria *M. testulalis* attacks 35 species of plants belonging to 20 genera and 5 families namely Papilionaceae, Caesalpinaceae, Pedaliaceae, Malvaceae, and Mimosaceae (Taylor, 1978). The majority of attacked plants belong to the Papilionaceae which includes *V. unguiculata* and *V. mungo* (Akinfenwa, 1975; Jackai and Daoust, 1986). *M. testulalis* has been reported as a pest of *Cajanus cajan, Crotolaria* spp,

and *Phaseolus vulgaris* in the following places: Fiji (Lever, 1944; 1947), Sri Lanka (Subasinghe and Fellows, 1978), The Philippines (Barroga, 1969). *M. testulalis* is a pest of groundnuts in Uganda (Jepson, 1948) while in Sierra Leone, it was reported to attack *Phaseolus vulgaris* (Hargreaves, 1937) and in Nigeria, Dina (1978) reported the insect to be a pest of Soya beans. In Egypt, Schmutterer (1968) described *M. testulalis* as a very important pest of the Egyptian bean *Vicia faba*. *M. testulalis* attack on *Phaseolus* beans in Africa is minor compared to other parts of the world like Asia, Latin America and Australia where this pest causes extensive damage on pods and leaves of *Phaseolus* beans (Barroga, 1969; Anon, 1978 and Broadley, 1977).

Control of this pest is complicated by its cosmopolitan life style which enables it to survive on a wide range of secondary host plants. During off season periods, the insect population is sustained on wild legumes, ornamental plants and market garden crops such as *Vigna triloba*, *Crotolaria juncea*, climbing beans (*Sphenostylis stenocarpus* L), Sunnhemp, winged beans, and wild pigeon peas (Taylor, 1967; 1978). These plants thus play important roles as alternative sources of food for *M. testulalis*.

Previous studies on *M.testulalis* focused extensively on its biology and damage potential to the principal host plant, the cowpea (Booker, 1963; Taylor, 1967; 1978; Usua and Singh, 1975; Singh, 1978; Singh and Van Emden, 1979; Odebeyi, 1981; Jackai, 1981b; Okeyo-Owuor and Ochieng, 1981; Okeyo-Owuor and Agwaro, 1982; Okech, 1986). Studies relating to feeding behaviour of *M. testulalis* have dealt with effects of feeding on yields. Suh and Simbi (1983) related qualitative plant damage to yield and loss assessment but no detailed studies on food consumption by the larvae were undertaken. Jackai and Singh (1983) investigated the development of *M. testulalis* larvae on the floral parts of selected leguminous plants and emphasis was placed on the nutritional potential of those parts. Otieno *et al.* (1985) showed that the chemical basis of TVU 946 resistance to *M. testulalis* feeding could be attributed to deterrent allelochemicals, but no specific compound(s) were identified. Den otter and Kahoro (1983) investigated the taste cell responses of *M. testulalis* mouthpart sensilla to NaCl, and sucrose but responses to the actual plant extracts were not tested. Okech (1986) demonstrated that cowpea cultivars were not equally acceptable to *M. testulalis*. The IITA cultivar, Vita 1, was found to be the most susceptible to *M. testulalis* infestation. Close examination of the above

studies show that there is no information on how host and non host plant characteristics influence the feeding behaviour of *M. testulalis*. Furthermore, no behavioural and electrophysiological biossays have been done using leaves, flower, pod and seed extracts. There is, therefore, a need to carry out both feeding and electrophysiological bioassays using known compounds as well as host and non-host plant extracts. Work reported in this thesis seeks to address some of these problems but it should be borne in mind that a chemist and the sensory biologist will have to work closely in order to identify the chemical basis of *M. testulalis* feeding behaviour.

The objectives of this study were to :

1. Examine feeding activity of first and fourth instar *M. testulalis* on host plants in relation to: Time of day, plant species, food deprivation, state of host plant, and developmental stage of the larva.
2. Examine food consumption by the fourth and fifth instar larvae of *M. testulalis*.
3. Carry out feeding bioassays using known compounds as well as host and non-host plant aqueous extracts.

4. Relate the feeding bioassay responses to the taste receptor sensitivity by employing electrophysiological bioassays using the same known compounds as well as host and non-host plant aqueous extracts.

These objectives emphasize the need for insect behaviour studies to become part of the integrated pest management (IPM) strategies (Odhiambo, 1987). It is expected that observations on feeding responses of *M. testulalis* will provide a basis for understanding the ecology of this pest in relation to its host and non-host plants. Cotton was chosen as a non-host plant because it is a non-legume that grows well in areas where cowpea and beans are grown. Its phenological and physiognomic development features also compare very well with those of host plants for *M. testulalis*. Furthermore, cotton is a host of various lepidopterous pests which include the cotton bollworm *Heliothis armigera*, spiny bollworm, *Earis biplaga*; pink bollworm, *Pectinophora gossypiella*; false codling moth, *Cryptophlebia leucotreta* and the red bollworm, *Diparopsis castanea* (Acland, 1971). Therefore, observations obtained should accord a good contrast between the two categories of plants and may also provide a basis for other workers to examine factors which make cotton a non-host for *M. testulalis*.

CHAPTER 2

LITERATURE REVIEW

2.1 Insect feeding patterns and factors that influence them

Insect feeding behaviour consists of sequence of activities which start with oriented movements followed by cessation of locomotion, palpating, test-biting and ingestion (Thorsteinson, 1960; Schoonhoven, 1968; Bernays and Simpson, 1982). During feeding, the insect usually follows certain patterns of responses which are expressed in terms of duration and frequency of components such as feeding bouts, locomotion and rest in between feeding bouts (Adler and Adler, 1988; Schmidt *et al.* 1988; Chapman and Beerling, 1990). Duration and frequency of feeding have been used as parameters to determine potential crop damage by the pest (Simmons and Yeargan, 1988) and the assessment of host plant resistance to pest attack (de Wilde, 1958). Locomotion and rests in between feeding bouts have been related to a search for a suitable feeding site while excessive locomotion has been related to the presence of host plant resistance characteristics (Beck, 1980; Adler and Adler, 1988; Schimdt *et al.* (1988). Feeding responses in phytophagous insects are

strongly influenced by insect species and age, plant characteristics, light regime and sensory inputs.

2.1.1 Insect species and age

Insect feeding responses differ from one insect species to another. Even within the same species, some differences in feeding responses due to age of the insect have been observed. Most of these results however have come from the study of locusts and grasshoppers and very little is known about Lepidopteran insects. In the generalist grasshopper, *Melanoplus sangiunipes* (Fabri.), feeding bouts are short (ca. 4-5 minutes) while in the specialist grasshopper, *Hypochlora alba* Dogde feeding bouts are relatively long (ca 10 minutes) accounting for only 4 % of the total insect activity (Blust and Hopkins, 1990). This contrasts with nymphal *Nomadacris septemfsciata* which spends 15-20 % of its activity on feeding. (Chapman, 1959). Among lepidoptera, larvae of *Philosamia cynthia* have feeding periods of about 15 minutes alternating with resting periods of about 50 minutes (Dethier, 1966).

2.1.2 Light regime

Environmental factors particularly light and temperature are known to have a direct influence on insect feeding behaviour (Bernays and Simpson, 1982;

Saunders, 1982). Many insects show certain feeding rhythms controlled by the diel cycle (Table 1). Depending on the time when the activity is maximal a diel periodicity may be termed diurnal when it occurs during day or light period (photophase), nocturnal when it occurs at night or dark period (scotophase) or crepuscular when it occurs at twilight (Corbert, 1966). Beck (1980) has defined a photoperiod as a cycle composed of light and dark periods in a 24 hour clock in reference to seasonal or lunar phenomena. The term circadian rhythm has been used for those insects where diel cycle is endogenously controlled (Saunders, 1982). Most of the insects in which the diel periodicity of feeding has been demonstrated are mainly Orthoptera and Diptera and very few studies on Lepidopterous larvae are known.

Table 1

Diel periodicity of feeding in some insect species

Insect	Order	Time of peak feeding	Reference
Aedes aegypti	Diptera	Late photophase	Gillet (1962)
Glossina morsitans	Diptera	Late photophase	Brady (1953)
Periplaneta americana	Orthoptera	Scotophase	Lipton and Sutherland (1970)
Schistocerca americana	Orthoptera	Late photophase	Chapman (1957; 1959)
Melanoplus sanguinipes	Orthoptera	Photophase	Blust and Hopkins (1990)
Hypochlora alba	Orthoptera	Photophase	Blust and Hopkins (1990)
Halisodota argentata	Lepidoptera	Scotophase	Edwards (1964a)
Nephytia phantasmaria	Lepidoptera	Scotophase	Edwards (1964b)
Orgyia pseudotsugata	Lepidoptera	Protophase	Edwards (1965)
Graphognathus leucoloma	Coleoptera	Scotophase	Senn and Brady (1973)
Graphognathus peregrinus	Coleoptera	Scotophase	Senn and Brady (1973)
Oncopeltus fasciatus	Hemiptera	Late photophase	Caldwell and Dingle (1967)

2.1.3 Plant characteristics

Plant characteristics influencing the feeding patterns of insects have been broadly classified as morphological and biochemical (Norris and Kogan, 1980). The morphological factors include physical features such as trichomes, cell wall thickness, texture, calcium and silica deposits while biochemical factors include primary and secondary plant substances incumbent in the plant tissues or on the surface (plant waxes). One or more of these factors may determine the level of resistance or susceptibility of plants to insect pest attack.

2.1.3.1 Morphological factors

Morphological factors affect mechanisms of feeding, attachment, locomotion, ingestion and digestion of plant materials. Early instars of the grasshopper, *Melanoplus*, are incapable of feeding on *Artemisia ludoviciana* due to the presence of trichomes (Smith and Grodowitzi, 1983). *Phaseolus* leaf trichomes trap and prevent feeding of *Aphis craccivora* (Johnson, 1953), *Acyrsiphon pisum* (Lampe, 1982), *Empoasca fabae* (Pillemar and Tingey, 1976; 1978) and *Heliconius* species (Denno and Donnelly, 1981). Our observations have also revealed that first and fourth instar *M. testulalis* are trapped by trichomes on the common bean

leaves. The resistance of some species of Lucerne (*Medicago spp*) to attacks by the weevil, *Hypera postica*, is attributed to the exudates of the glandular trichomes which prevent it from moving and feeding (Levin, 1973). Larvae of the sphingid moth, *Pterogon proserpina* Pallas, cannot eat mature leaves of their host plant *Vitis* because they have accumulated bundles of needle shaped calcium oxalate crystals which prevent larval biting (Merz, 1959). These are just but a few examples showing how plant morphological factors affect the feeding responses of insect pests.

2.1.3.2 Biochemical factors

It is now widely accepted that both primary and secondary plant chemicals are important factors in regulating insect feeding behaviour (Hsiao and Fraenkel, 1968a; Hsiao, 1969). Many of these compounds act as phagostimulants (Table 2) but others are feeding deterrents (Table 3). Phagostimulants include primary compounds and secondary plant substances. Primary compounds are essential to the development of insects and include chemicals such as sugars, amino acids, inorganic salts, vitamins and phospholipids. Secondary substances take no active part in the metabolic processes of the plant but are used to

signal the presence of food. Components of the surface waxes belong to this group and their role has been shown in a number of insects. The alkane fraction of *Vicia faba* causes settling and feeding by *Acyrthosiphon pisum* (Klingauf *et al.* 1978). The apple aphids, *Rhopalosiphon insertum* and *Aphis pomi*, are stimulated to probe by glucoside phlorizin in apple leaf wax (Klingauf, 1971). In phytophagous insects, duration of feeding on the host plant has been linked to the presence of phagostimulants and absence of feeding deterrents (Mulkern, 1969). Thus, on the susceptible potato, *Solanum tuberosum*, larvae of the Colorado potato beetle, 90 % of their activity is feeding and the remaining is shared between resting and wandering. On the resistant *Solanum demissum*, 26 % of the insect activity is feeding while 74 % is resting (de Wilde, 1958).

Deterrents are secondary plant substances which, when tasted by the insect, induce cessation of feeding either temporarily or permanently depending on their potency (Nakanishi, 1977). Some deterrents like pyrethrins, nicotine and rotenoids are toxic to many insects (Bell, 1986). Other deterrent secondary plant substances are produced in the plant foliage following wounding leading to a reduction in the acceptability of the plant to feeding. This phenomenon has been

linked to defence mechanism of the plant (Rhoades, 1979; Edwards and Wratten, 1985). Green and Ryan (1974) showed that wounding of the leaves of the tomato led to accumulation of a proteinase inhibitor that was linked to plant defence. Haukioja and Niemela (1977) showed that the growth of larvae of the geometrid moth, *Epirrita autumnata*, was adversely affected if they were fed either on grazed leaves of *Betula pubescens* spp.*tortuosa* or nearby leaves of the same plant. Recently, Wratten *et al.* (1984) showed that *Spodoptera littoralis* and *Orgyia antiqua* larvae found damaged leaves and those nearby on the same plant less acceptable for feeding 6 hours following artificial damage. It was concluded that physical damage to the leaf led to changes in the chemistry of secondary compounds that reduced food.

Table : 2

Examples of phagostimulants for some insect species

Insect	Chemical	Reference
Pieris brassicae	Mustard oil glycoside	Verschafelt (1910)
Ostrinia nubilalis	Sucrose	Beck (1956)
Otiorhynchus sulcatus (F.)	Sitosterol	Shanks and Doss (1987)
Choristoneura fumiferana	Glucose	Albert *et al.* (1982)
"	Fructose	"
"	Inosital	"
Delia antiqua (Meigen)	Fructose	Mochizuki *et al.* (1985)
Dysdercus koengii	Raffinose	Hedin *et al.* (1977)
Eureme hecabe Mandarin	Pinitol	Numata *et al.* (1979)
	Fructose	"
	Myoinositol	"

cont..d

Nilaparvata lugens (Stal)	Asparagine	Sogawa (1982)
Ostrinia nubilalis	Serine + Glucose	Beck and Hanec (1958)
Myzus persicae (Sulzer)	Methionine and Sucrose	Mittler and Dadd (1964)
	Leucine and sucrose	"
Choristoneura fumiferana	Sucrose + L-proline	Albert and Jerret (1981)
"	Sucrose + L-hydroxyproline	"
"	Sucrose + L-glutamic acid	"
"	Sucrose + L- arginine	"
Chortihippus curtipennis	Betaine	Dethier (1966)
	Ascorbic acid	"
	Thiamine	"
Sitona cylindricolis	Adenosine	Hedin *et al.* (1977)
Leptinotarsa decemlineata	Phospholipids	Hedin *et al.* (1977)

cont..d

Otiorhynchus sulcatus (F.)	Sitosterol	Shanks and Doss (1987)
Sciopithes obscurus (Horn)	"	"
Hypera postica	"	"
Plutella maculipennis (L)	Sinigrin	Whittaker and Feeney (1971)
Phylotreta cruciferae Goez	Glucaparin	"
Phylotreta striolata	Glucoiberin	"
Plutella maculipennis	Glucosinolates	Nayar and Thorsteinson (1963)
Leptinotarsa decemlineata	Chlorogenic acid	Hsiao and Fraenkel, (1968b)
Diabroticine beetles	Curcubitacins	Chambliss and Jones (1966)
Mexican beetle	Phaseolutin	Hedin *et al.* (1977)
	Lotaustrin	"
	Linamarin	"
Junonia coenia	Catapol	Bowers (1983)

cont..d

Scolytus multistriatus	p-hydroxybenzaldehyde	Baker *et al.* (1968)
Gonioctena vitallinae	Salicin	Hutchison (1931)
Phyllodecta vitallinae	Salicin	"
Chrysomellia vigontipunctata	Populin	Matsuda and Matsuo (1985)
	Luteolin-7-glucoside	"
Bombyx mori	B-sitosterol	Hamamura *et al.* (1962)

Table: 3

Examples of feeding deterrents for some insect species

Insect	Chemical	Reference
Epicauta spps	Gossypol	Maxwell *et al.* (1965)
Ostrinia nubilalis	Dimboa	Klun *et al.* (1967)
Spodoptera exempta	Warbuganal	Kubo *et al.* (1976)
Cnaphalocrosis medinalis	Azadirachtin	Saxena *et al.* (1981b)
Nilaparvata lugens	Azadirachtin	Saxena *et al.* 1981a
Empoasca fabae	Leptin-I	Dahlman and Hibbs (1967)
Bombyx mori	Strychnine	Schoonhoven (1982)
Mamestra brassicae	Strychinine	Schoonhoven (1982)
Scolytus multistriatus	Juglone	Gilbert *et al.* (1967)

cont..d

Myzus persicae	Sinigrin	David and Gardner (1966)
Epicauta spp	Coumarin	Gorz *et al.* (1972)
Myzus persicae	Phlorizin	Montgomery and Arn (1974)
Amphorophora agatonica	Phlorizin	Montgomery and Arn (1974)
Sitona cyrindricollis	Ammonium nitrate	Akeson *et al.* (1969)

2.1.4 The Sensory system

Food selection by lepidopteran larvae is aided by their sensory systems located in the antennae, maxillae and the epipharnyx. These systems include taste, odour and tactile sensitive cells. Each antenna usually bears three sensilla basiconica innervated by 16 odour sensitive neurones. Each maxilla bears one palp and a galea. The galea is equipped with two sensilla styloconica within an apical papilla. Each styloconicum sensillum is usually innervated by four bipolar neurones directly wired to the central nervous system. The dendrites of these neurones communicate with the external world through a pore at the tip (Ishikawa, 1963; Ishikawa and Hirao, 1963; Ishikawa, Tazima and Hirao, 1963; Schoonhoven, 1986). The maxillary palp usually has eight basiconica sensilla innervated by a total of 14-19 neurones (Schoonhoven and Dethier, 1966). Some of the sensilla are multiporous signifying olfactory function and the others are typical taste sensilla with a single pore at the tip (Dethier and Kuch, 1971; Schoonhoven, 1972; Hanson and Dethier, 1973). A pair of gustatory organs also known as epipharyngeal organs are located on the epipharynx and are usually innervated by 3 neurones (Ma, 1972). The epipharyngeal sensilla are known to play roles similar to those of the styloconica

sensilla (Dethier, 1975; Ma, 1976; de Boer *et al.* 1977; Drongelen, 1979; Albert, 1980). In some larvae, however, they are missing as in *Mamestra brassicae* and *Euxoa messoria* (Blom, 1978; Devitt and Smith, 1982). The antennal maxillary palp and the epipharyngeal organs serve different functions. Experimental work in which these organs have been inactivated in various combinations have shown that the sensilla styloconica and the epipharyngeal organs are the most critical receptors in the process of distinguishing host from non-host plants (Torii and Morii, 1948; Dethier, 1953; Ito *et al.* 1959; Waldbauer and Fraenkel, 1961; Schoonhoven and Dethier, 1966; Ma, 1972; deBoer *et al.* 1977; Blom, 1978; Remorov, 1982; Schoonhoven, 1986).

More electrophysiological bioassay work has been done on sensilla styloconica than the epipharyngeal organs. In *Pieris brassicae*, the 4 cells of the medial styloconica sensillum on the maxillae are sensitive to salt, strychnine, sucrose and sinalbin. The lateral styloconic sensillum has cells sensitive to sucrose sinigrin, proline and anthocyanins, while the epipharyngeal organs respond to sucrose strychnine and salt (Ma, 1972). Examples in other lepidopteran larvae are given by Dethier and Kuch, (1971).

2.2 Influence of the rearing food plant on the feeding preferences of the penultimate larvae

Larval food during early instars has been shown to influence the feeding preferences of penultimate instars in various phytophagous insects (Jermy *et al.* 1968; Dethier and Crnjar 1982; Papaj and Prokopy, 1989). Some of the effects of the rearing food plant on feeding preferences include: Induction of feeding preference, habituation, aversion learning, and sensitization (Papaj and Prokopy, 1989).

Induction of feeding preference has been defined by Jermy *et al.*, (1968) and Dethier and Crnjar, (1982) as a process whereby ingestion of a particular food during ontogeny creates a preference for that food over others previously more acceptable. This has been shown in a number of oligophagous and polyphagous species belonging to the orders: Phasmida, Hemiptera, Coleoptera and Lepidoptera (Jermy, 1987).

Induction of feeding preference has been shown in several lepidoptera: In *Manduca sexta* (Johansen), Jermy *et al.* (1968) and Hanson and Dethier (1973) showed that larvae raised on tomato prefer it in a choice test whereas animals raised on *Solanum* prefer it an a choice test. In a separate study using the same insect, Stadler and Hanson (1978) showed that

rearing of larvae on leaves added to artificial diet or homogenized leaves added to an artificial diet induced a preferences for that food in subsequent choice. Wasserman (1982) further showed that the strength of induction reflects the degree to which 2 hosts are sensorially distinguished by the insect and demonstrated that induction was strongest when the 2 host plants are phenotypically distinct to the larvae. Ting (1970) suggested that absence of induction is plant specific and certain hosts are unable to alter the preference of certain insects as shown in *Pieris napi* (Chew, 1980). This illustrates that, providing lower instar larval stages with food from plants not usually fed upon can affect subsequent feeding preferences of the penultimate larval stage of some lepidopteran larvae.

CHAPTER 3

GENERAL MATERIALS AND METHODS

3.1 Insect source

Larval stages of *Maruca testulalis* Geyer (Lepidoptera: Pyralidae) were used throughout this study. They were obtained from the ICIPE colony reared on a natural cowpea diet using the methods developed by Okeyo Owuor and Ochieng (1981).

3.2 Plant materials

Two host plants belonging to the Leguminous family namely: cowpea (*Vigna unguiculata* (Walp) cultivar Vita 1, an IITA cultivar), common beans (*Phaseolus vulgaris* (L.) variety Rosecoco) and a non-host plant cotton (*Gossypium hirsutum* (L.)) were grown both in the screenhouse and field plots. In the screenhouse, seeds were sown either directly in the soil (Plate 2) or in 5 litre buckets filled with rich garden soil (Plate 3). At germination only healthy seedlings (3 for cowpea and beans and 2 for cotton) were allowed to grow and the rest were all thinned out. Planting was done at a 2 week interval throughout the experiments. This made it possible to have plants of the required age all the time. In the field, 3 plots (2.5 x 3.0 metres) were used for growing plant

Gustatory cells in the lateral sensilla were more sensitive to host and non-host plant extracts, sucrose, methionine and glutamine than those in the medial sensilla. The spike frequency generated as well as the feeding responses observed were concentration-dependent. This implies that inputs from the lateral sensilla gustatory cells play a vital role on host plant recognition. Those cells are well tuned to detect the presence of primary compounds such as sugars and amino acids. On the other hand, aqueous extracts from cotton leaves and flowers evoked different spike patterns from the lateral sensilla gustatory cells. Since these patterns were associated with non feeding responses it implies that aqueous extracts from the non-host plant either acted on different sensory cells or modified the responses of those cells sensitive to the phagostimulants in the host plant extracts.

ABSTRACT

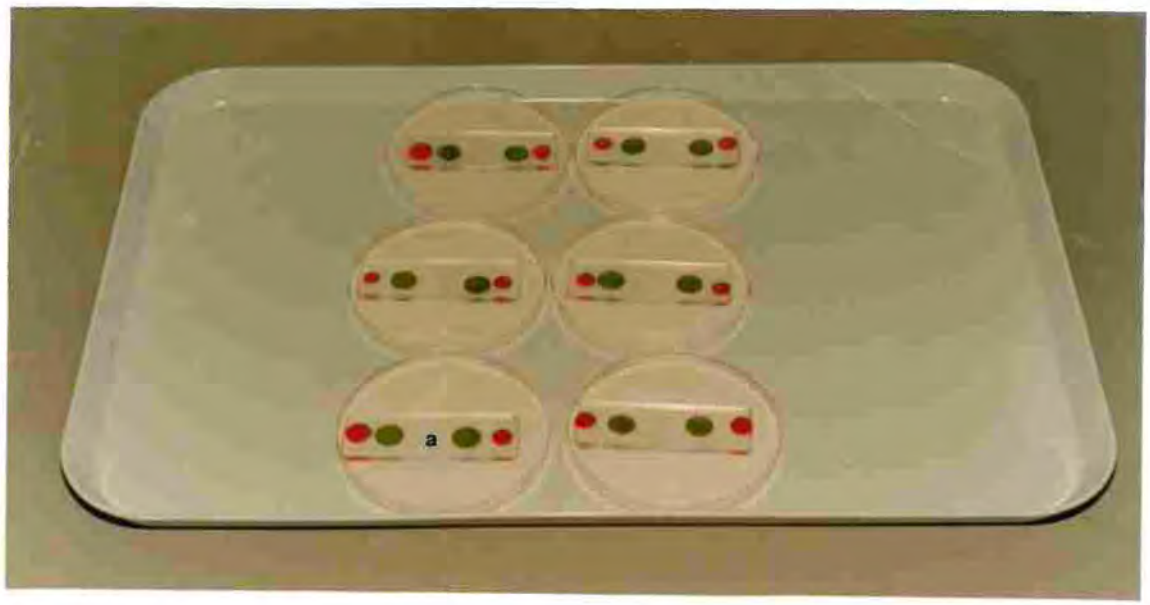
The feeding behaviour of first, fourth and fifth instar larvae of the cowpea pod borer *Maruca testulalis* (Geyer) (Lepidoptera:Pyralidae) on the host plants cowpea (*Vigna unguiculata* (Walp) cultivar Vita 1), and common bean (*Phaseolus vulgaris* (L.) var. Rosecoco) and the non-host plant cotton (*Gossypium hirsutum* (L.)) was investigated.

Feeding responses of the first instar larvae on the host and non-host plant leaves, flowers and pods (cotton bolls) comprise a series of feeding bouts alternating with rest or locomotion. Long durations and relatively high frequency of feeding were observed on the host plant flowers and pods while longer rest periods were observed on host plant leaves. Long feeding bouts were generally associated with long locomotory bouts. The length of feeding bouts on the non-host flowers and leaves were similar to those observed on the host plant flowers and leaves. However, larvae rested for relatively longer periods between successive feeding bouts on the cotton leaves and spent a greater proportion of time in locomotion on the cotton flowers.

Feeding responses exhibited by the fourth instar *M. testulalis* varied with the part of the plant presented. Feeding bouts were much shorter on the cowpea leaves than on the cowpea flowers and pods while the rest periods were much longer on the leaves than on flowers and pods. This was an indication that the host plant flowers and pods may have the optimum phagostimulant composition.

Time of day had no influence on larval feeding patterns and quantity of food intake among larval instars tested. This means that larval feeding responses are not under diel rhythms of light and darkness.

Presenting excised cowpea leaves to the first instar larvae reduced feeding duration. However, feeding duration on excised flowers and pods was similar to that of flowers and pods on intact plants. This implied that excision of these parts did not bring about any such change in their physical or chemical characteristics that could affect the insect's feeding. Hence, the observations of the responses to the excised plant parts could serve as valid basis for drawing conclusions.



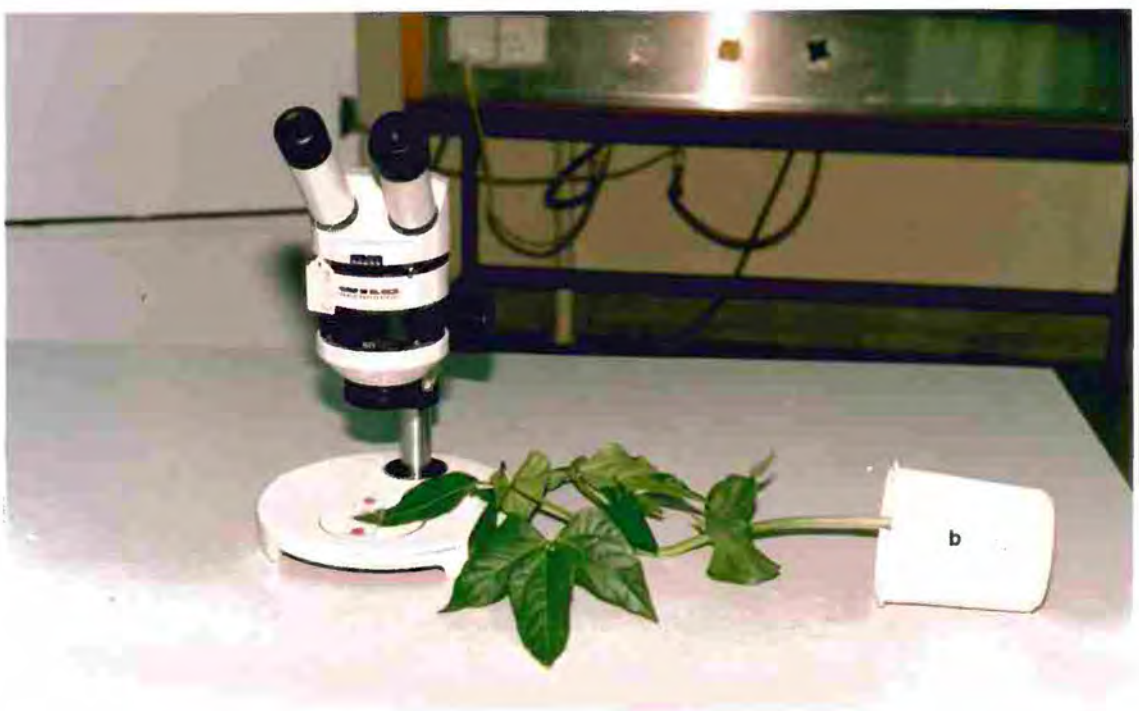


Plate 6a: Leaf discs mounted in between cavity slides and held together by placiticine (red).

b: Potted experimental plants used to examine larval feeding responses on intact plants.

the effect of food deprivation on feeding responses of *M. testulalis* larvae was also investigated.

4.2 Materials and methods

4.2.1 Feeding responses of the first and fourth instar larvae under various conditions

Feeding responses in these studies were carried out on 2-3 week old cowpea, common bean or cotton leaves and on the flowers. A cork borer No 8 was used to punch out 1 cm discs. Each disc was put in a cavity slide (No. 3720, size 7.5 x 2.5 cm). Two newly emerged 1st instar *M. testulalis* were placed on top of each leaf/flower disc and immediately covered with a second cavity slide. A little placiticine was placed in between the slides to keep them in position (Plate 6a). This arrangement allowed free movement of the larvae and prevented rapid wilting of the discs. The larval feeding behavioural responses were observed with the aid of a Wild Heerbrug M5A dissection microscope. During the day, these observations were made using natural light in the laboratory while at night, red light was used. Three periods of the diel cycle were selected and these were: 08.00 - 12.00 hrs, 14.00 - 18.00 hrs and 20.00 - 24.00 hrs.

3.4.4 Preparation of insect saline and electro -conductive solutions

Beadle's solution was used as the physiological saline in these studies. It was made by dissolving 7.5gm NaCl, 0.35gm KCl, 0.29gm $CaCl_2.H_2O$ and 0.22gm $CaCl_2$ in 100ml double distilled water. The electro-conductive electrolyte used in these studies was 50 and 100mM NaCl. The 50 mM NaCl solutions was used to dissolve pure compounds while plant extracts were dissolved in 100 mM NaCl.

3.4.5 Preparation of recording electrodes.

Recording electrodes were made from silver wire. AgCl was heated in a crucible with a gas flame till it melted to liquid of amber colour. The silver wire was dipped into the molten AgCl to obtain Ag/AgCl wire required for electrophysiological recordings (Hodgson *et al.* 1955).

3.4.6 Pulling of the glass micropipettes

Glass micropipettes were pulled from Microhaematocrit capillary glass tubes (1.1- 1.2 mm inner diameter, and 1.5-1.6 mm outer diameter) (Propper manufacturing company) using a DK1 vertical pipette puller, Model 700C (David Kopf Instruments)

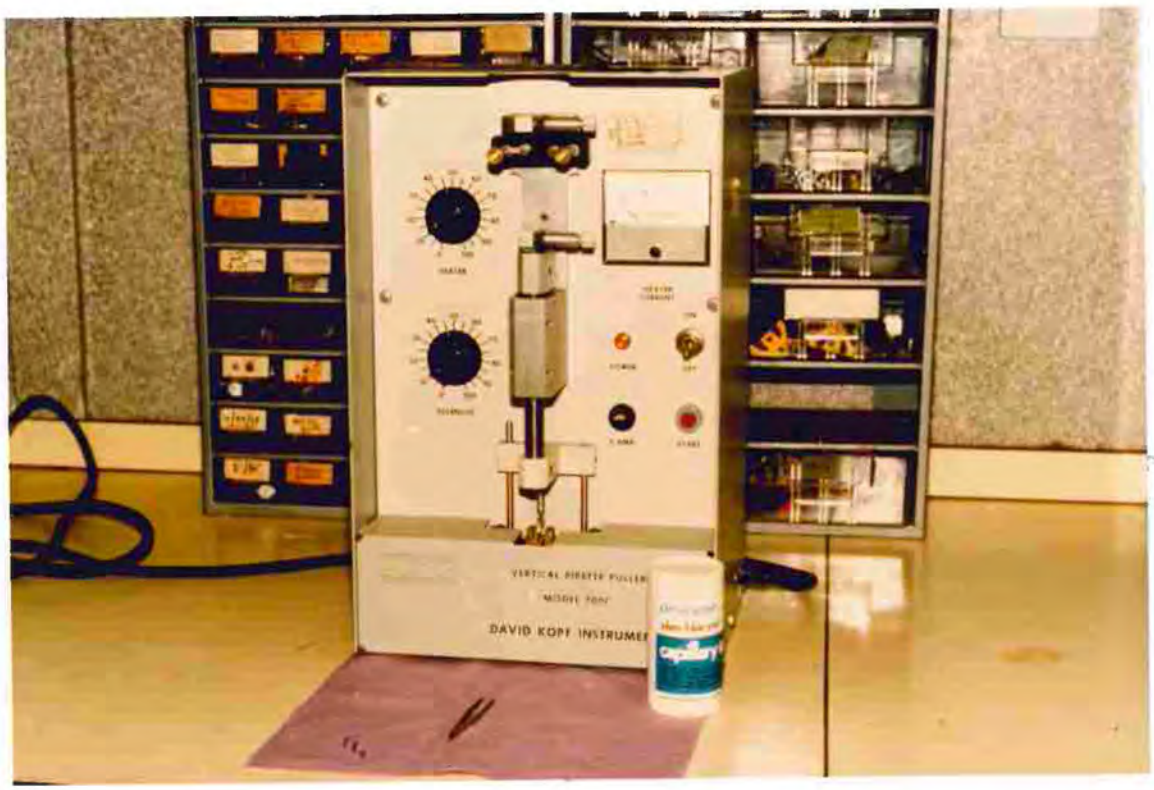


Plate 5: A DK1 vertical pipette puller for making glass micropipettes.

(Plate 5). The puller heater was adjusted so as to deliver a current of 18 amperes while the solenoid force settings were adjusted to 45 units. This gave adequate force that enabled the preparation of suitable pipette tips.

3.4.7 Recording instrumentation

The recording set-up consisted of 2 amplifiers connected in series. A Grass P16, the primary preamplifier, was fitted with Ag/AgCl microelectrodes, one serving as the reference electrode and the other as a recording electrode. Signal output from this preamplifier was fed into a P15 grass preamplifier. This was in turn connected to the oscilloscope, a Racal Store 4DS magnetic tape recorder, camera and the audiomonitor. The recording stage was set up in a Faraday's cage. All components in the cage were grounded to eliminate unwanted noise and improve the signal to noise ratio. Results of the feeding and electrophysiological bioassays are discussed in chapter 7.

CHAPTER 4

FEEDING RESPONSES OF FIRST AND FOURTH INSTAR *M. TESTULALIS* LARVAE IN RELATION TO TIME OF DAY, PLANT SPECIES, FOOD DEPRIVATION AND DEVELOPMENTAL STAGE OF THE INSECT

4.1 Introduction

It is known that feeding responses in some lepidopterous larvae are under the influence of light and darkness cycle, plant species, state of host plant, food deprivation and developmental stage of the insect. Under this broad title, several aspects of the feeding behaviour of *M. testulalis* were investigated.

1. In order to compare the feeding responses on different parts of the host plants, it was necessary to establish if light and darkness cycle influences the feeding pattern and food intake. This would be quite useful in choosing the appropriate time to investigate feeding responses on host and non-host plants.

2. Biochemical changes are known to occur in the plant foliage after damage or separation from the

parent plant (Green and Ryan, 1972, 1974; Haukioja and Niemela, 1977; Tapper and Reay, 1973). Some of those changes are associated with the plant defence system against pest attack. This defensive response can reduce feeding but in other cases, reduced feeding may merely be due to loss of water in the excised tissues (Scriber, 1979). It is not yet known whether the feeding responses of *M. testulalis* larvae on excised parts of its host plants are similar to those on the intact plants.

3. *M. testulalis* larvae do not feed on cotton in the field in spite of the fact that farmers usually intercrop it with cowpea and common beans and shares many physiognomic and phenological similarities with these legumes. Since it is not known why this pest avoids feeding on cotton, this aspect was investigated.

4. Newly emerged lepidopteran larvae are known to have different feeding preferences when compared with older instars. *M. testulalis* oviposits mainly on host plant leaves and this is where the early instars feed. But as they grow older, they prefer to feed on flowers and pods. A comparison in the feeding responses of the first instar and fourth instar larvae on cowpea parts was carried out. In addition to the foregoing,

Plate 4a: A virtis® freeze drier with freeze drying bottles containing plant extracts.

b: Labconco® freeze drying bottles containing frozen extracts before attachment to the freeze drier.

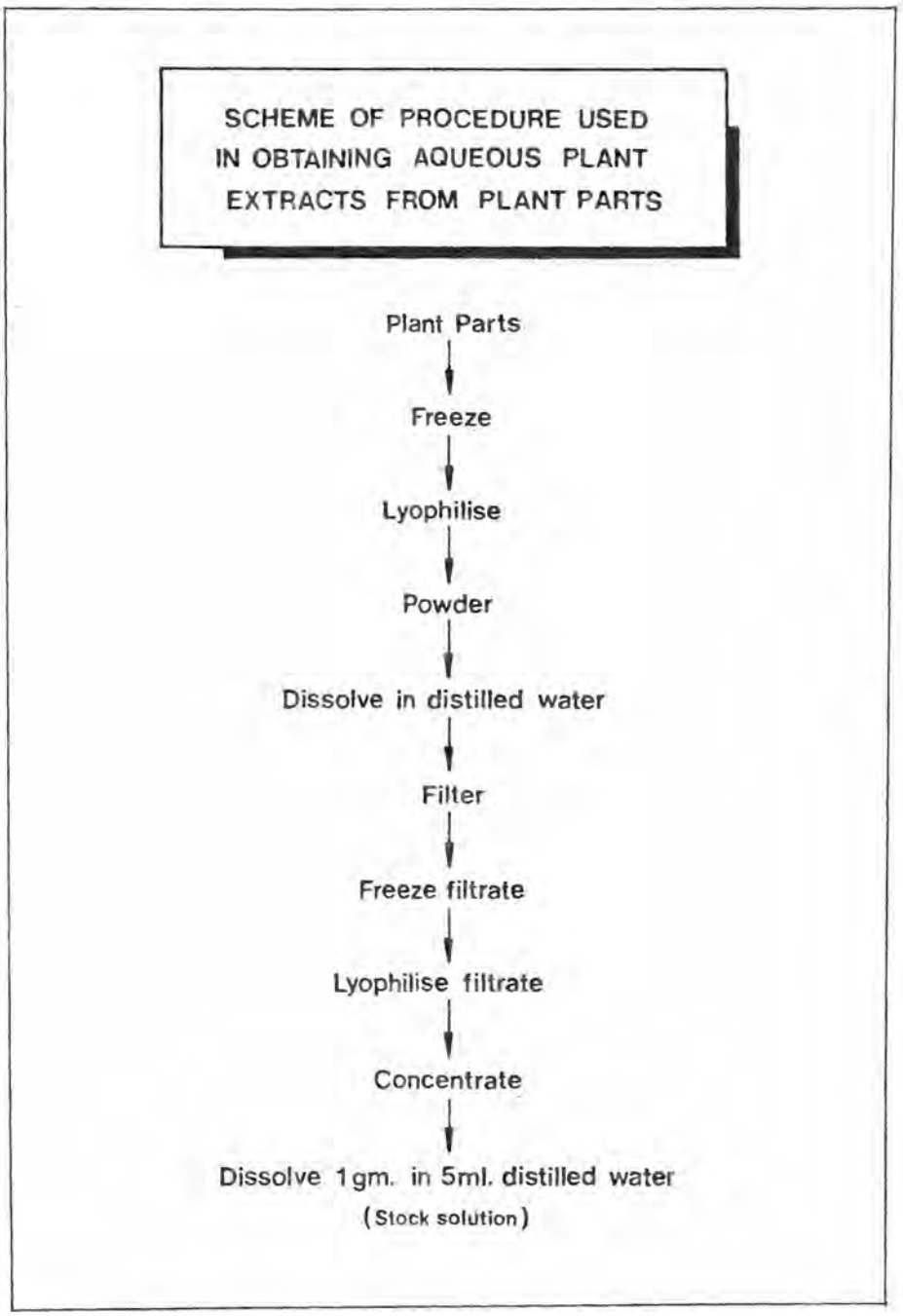


Figure 1: Scheme for preparation of plant extracts used in feeding and electrophysiological bioassays.

materials used in preparation of plant extracts. No herbicides or pesticides were used but NPK fertilizers were applied at the time of planting at the rate of 40kg/ha.

3.3 Behavioural studies

Experiments were undertaken to investigate the following aspects:

(1) Feeding responses of the first and fourth instar larvae in relation to time of day.
(2) Feeding responses of the first instar larvae in relation to condition of the host plant (excised versus intact plant material).
(3) Feeding responses of first instar larvae on the non-host plant.
(4) Feeding responses of the first instar larvae in relation to food deprivation.
(5) Food intake in fourth instar larvae on host and non-host plant parts in choice and no choice situations.
(6) Effect of host and non-host plant parts on larval growth and development.
(7) Role of larval rearing food on the feeding preferences of the fifth instar *M. testulalis* larvae.

(8) Feeding response of fourth instar larvae on host and non-host plant extracts and known phagostimulants (amino acids and sugars) incorporated in agar cellulose gels or cellulose acetate discs.

(Results of experiments 1-4 and 5-7 are discussed in chapters 4 and 5 respectively, while the results of experiment 8 are discussed in chapter 6).

3.3.1 Site

The above investigations were conducted at ICIPE's Mbita Point Field Station (M.P.F.S), situated on the south eastern shores of lake Victoria in South Nyanza Province of Western Kenya. The station stands at an elevation of 1240 metres above sea level, at latitude 0° 30'South and longitude 34° 15'East. The area receives a mean annual rainfall of about 900 mm which is distributed in two peaks, one starting in March to May and the second one in October and November. Since these rains are unreliable and erratic, irrigation of the plots was done whenever necessary.

3.3.2 Laboratory conditions

Laboratory temperatures ranged from 22°-28°C with a relative humidity of 40-60 %. Observation were made on a laboratory bench under fluorescent light. Observation at night were made with a lamp fitted with a red filter.

3.4 Feeding and electrophysiological bioassays

Feeding and electrophysiological bioassays were carried out using known compounds as well as aqueous extracts from host and non-host plants. The experimental animals used in this work were fourth and fifth instars of *M. testulalis* larvae.

3.4.1 Site

Both bioassays were carried out at the ICIPE International Headquarters at Duduville in Nairobi. All the work was done using facilities in the Sensory Physiology Research Unit.

3.4.2 Laboratory conditions

Laboratory temperatures ranged from 20-26°C with a relative humidity of about 40%-60%. Temperatures below 23°C were found unsuitable for recording

purposes and extra heat was supplemented with a Phillips heater.

3.4.3 Preparation of plant extracts

Plant parts were harvested and immediately loaded in Virtis® freeze drying bottles and kept in a freezer before use. The bottles containing frozen plant materials were connected to a Virtis freeze drier (Model No. 10-030) (Plate 4a) and freeze-dried thoroughly. The materials were then ground into powder and extracted with limited volumes of water (Fig. 1). The extract was filtered at 0°C with Whatman filter paper No.1 and frozen in Virtis® freeze drying bottles (Plate 4b). The frozen extract was freeze dried to obtain fine powder. A 200 ug/ul stock solution of the extract was made by dissolving 1g of the extract powder in 5ml of double distilled water. Serial dilutions of the stock solution was made to obtain solutions of 100, 50, 25, 12.5, 6.25, 3.25, 1.5 and 0.78ug/ul.

Plate 3: Common bean plants growing in a bucket.

Plate 2: Screenhouse grown experimental plants (a) Cotton (b) Cowpea.

Plate 8: Sandwhich boxes in which larvae were reared.

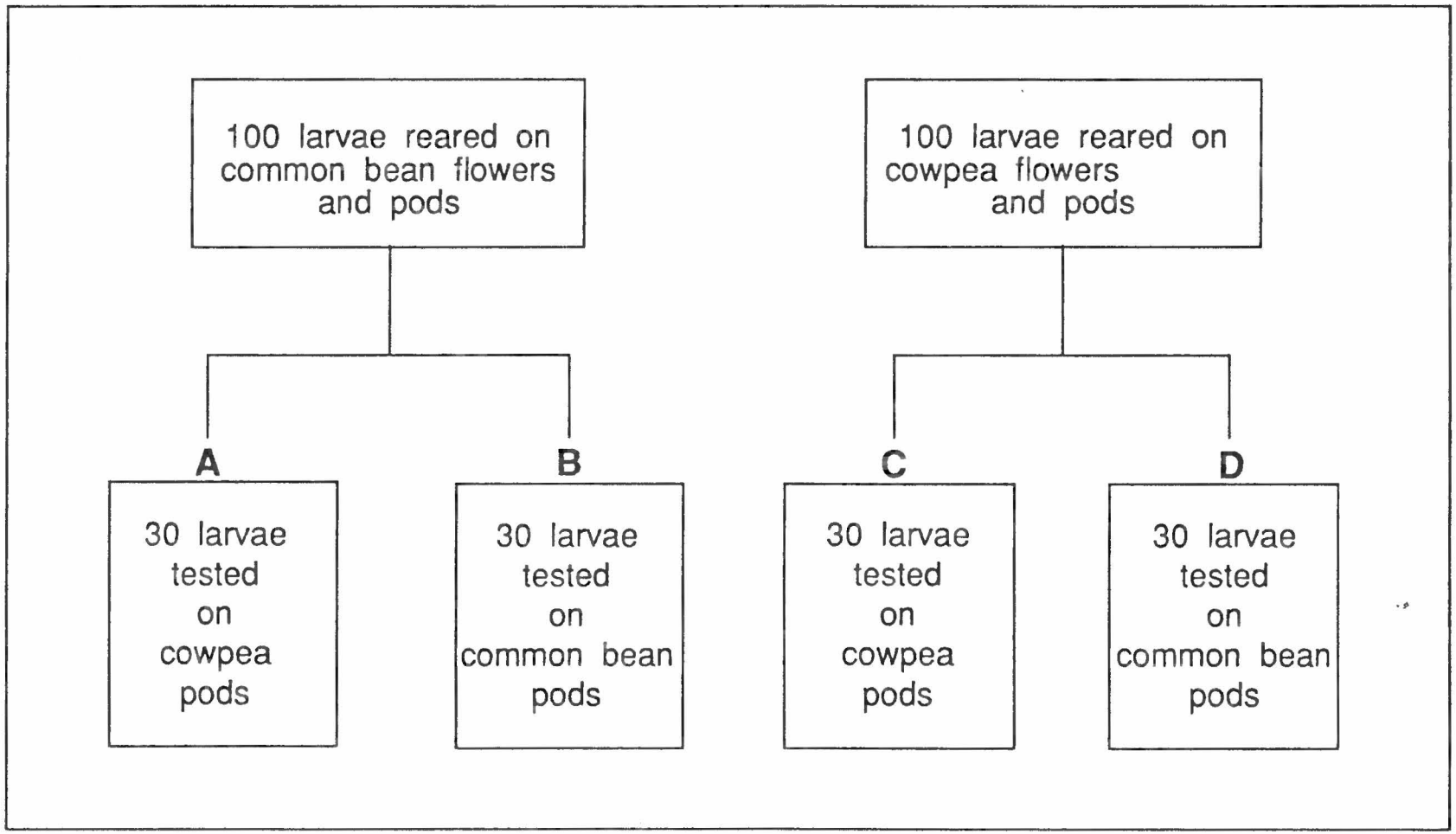


Figure 2: Scheme for studying the role of larval rearing food on feeding preferences of the fifth instar *M. testulalis* larvae.

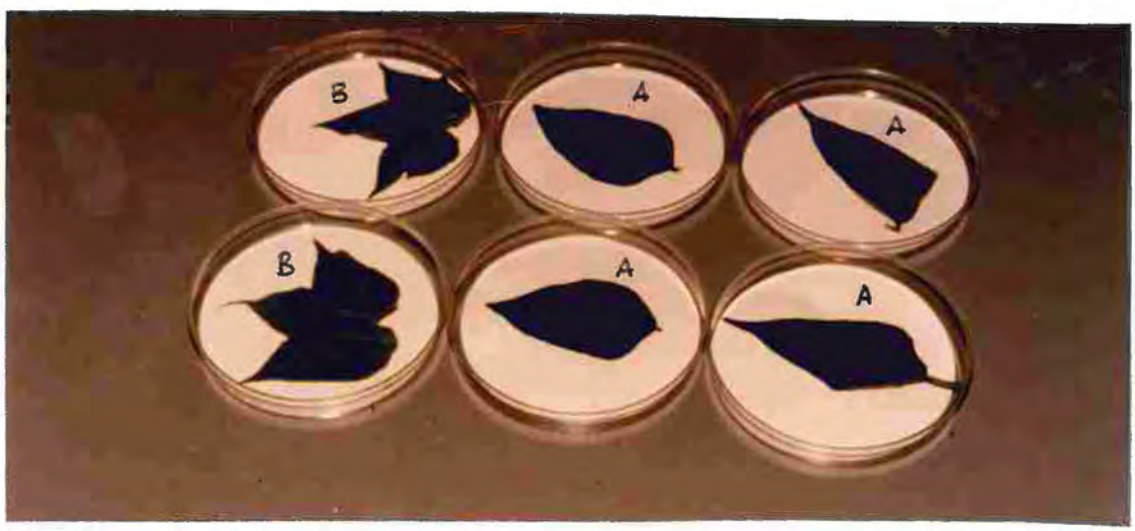


Plate 7: Host and non-host plant parts placed in plastic Petri dishes for food consumption studies.

A : Host plants

B : Non-host plant

4.2.2 Influence of time of day on food intake in fourth instar *M. testulalis* larvae

Fourth instar larvae were sorted out according to their head capsule measurements (Odebeyi, 1981). Larvae were immobilised under ice and width of the head capsule was measured with an ocular micrometer graticule fitted in the eye piece of a Wild Heerbrug M5A microscope. Selected larvae were starved for about 10 hours to enable them empty their guts and placed singly in plastic Petri dishes (6cm diameter) lined with a wet filter paper that provided moisture (Plate 7). Test plant materials comprised freshly excised 2-3 week old leaves, and stem, freshly opened flowers and 10-15 days old developing pods (pericarp) and developing seeds of cowpea and common beans. Each test material was weighed using a Sartorius digital balance (Type 1712) to obtain its initial weight (D1) and placed in the Petri dish containing one larva . Different batches of larvae were fed separately for 12 hours of day light starting at 7.00 am and 12 hours of darkness starting at 7.00 pm (LD 12:12). After feeding, each test material was removed from the dish and faecal pellets carefully removed. The uneaten plant material was weighed to obtain the weight of the eaten plant material (D2). In the control experiments, no larvae were put in the Petri dish. However, its initial (C1) and final (C2) weights were

A modification of the set-up was devised to monitor the feeding patterns of the fourth instar larvae. Since these are fairly big larvae, they were placed in small test tubes measuring 6 cm long by 0.9 cm diameter. Freshly excised parts of cowpea leaf, flower or pod were inserted into the tube and it was then plugged with some cotton wool to prevent the larvae from escaping. This set-up also enabled the larva to conceal itself while feeding thus simulating the natural environment in which the fourth instar larvae feed.

Feeding patterns on intact plants were studied on plants grown in small plastic containers (Plate 6b). The pots were placed either vertically or horizontally on a laboratory bench to enable manipulation of the desired leaf or flower into the appropriate orientation. The selected leaf or flower was clamped under the microscope stage with placiticine to minimise mechanical injury to the plant. Observations on feeding responses were made by placing the insect in a feeding arena made on the leaf or flower on the intact plant. The arena was constructed by making a 1 cm diameter, 2 mm high ring with 6% cold agar in water. A glass slide was then placed over the agar ring in order to confine the larva in the arena. The agar ring also ensured that the leaf or flower

tissue was not bruised during the experimental process.

In the experiment to investigate the effect of food deprivation on feeding responses, first instar larvae were starved for 1, 2, 3 or 4 hours. Controls comprised larvae that were not starved. In a related experiment, feeding responses were observed continuously over a 2 hour period divided into 4 treatments of time, each lasting 30 minutes.
All observations in the above experiments lasted 30 minutes using different animals at different times.
Parameters measured included:

Total duration of feeding (TDF),
Number of feeding bouts (NFB)
Average duration of a feeding bout (AVDF)
Total duration of movement (TDM)
Number of movement bouts (NMB)
Average duration of a movement bout (ADMB)
Total duration of rest (TDR)
Number of rest bouts (NRB)
Average duration of a rest bout (ADR)

Locomotion in between feeding bouts involved all those movements made by the larvae including sideways movement of the anterior part of the insect when it was stationary. Resting constituted the time in between feeding bouts or locomotory bouts when the larva was neither feeding nor moving. Total time spent

on feeding was determined by adding up the time in minutes that the larvae spent on feeding which consisted of continuous biting movements usually lasting approximately 30 seconds. Feeding frequency was obtained by counting the feeding bouts while duration of a feeding bout was derived by dividing the total time spent feeding with the number of feeding bouts during that observation period. Locomotion and rest activities in between feeding bouts were measured in the same way. Each experiment had 10 replicates and data were analyzed as completely randomized design (C.R.D). Data on the influence of the time of day on feeding responses of the larvae on its host plant parts were analysed by the analysis of variance procedure (ANOVA) and means were separated by the Duncan's Multiple Range Test (D.M.R.T) (SAS Institute, 1985). To test differences in feeding responses on excised and intact host plant materials, and feeding responses between first and fourth instar larvae, a t-test procedure for paired comparisons was used.

Table 9

Feeding responses of first instar *M. testulalis* on excised and intact (attached) cowpea leaves.

Parameter measured	Plant part offered: Excised Mean ± S.E	Attached Mean ± S.E	T	P< 0.05
Total duration of feeding (min/30min)	16.7 ± 1.44	21.9 ± 0.95	0.007	SIGN
Number of feeding bouts (No/30min)	4.80 ± 0.32	4.70 ± 0.42	0.853	NS
Length of a feed bout (min/30min)	3.66 ± 0.46	5.04 ± 0.53	0.068	NS
Total duration of locomotion (min/30min)	5.23 ± 1.17	7.05 ± 0.96	0.247	NS
Number of locomotory bouts (No/30min)	2.60 ± 0.42	3.80 ± 0.32	0.030	SIGN
Length of a locomotory bout (min/30min)	2.15 ± 0.33	2.00 ± 0.32	0.750	NS
Total duration of rest (min/30min)	8.12 ± 1.73	1.15 ± 0.69	0.001	SIGN
Number of rest bouts (No/30min)	2.90 ± 0.58	0.70 ± 0.39	0.006	SIGN
Length of a rest bout (min/30min)	2.23 + 0.41	0.28 ± 0.19	0.0004	SIGN

N=10 for each parameter

Table 10

Feeding responses of the first instar *M. testulalis* larvae on excised and intact (attached) cowpea flowers.

Parameter measured	Plant part offered: Excised Mean ± S.E	Attached Mean ± S.E	T	P< 0.05
Total duration of feeding (min/30min)	21.6 ± 0.76	20.9 ± 1.35	0.657	NS
Number of feeding bouts (No/30min)	5.30 ± 0.36	3.60 ± 0.30	0.002	SIGN
Length of a feed bout (min/30min)	4.35 ± 0.47	6.25 ± 0.80	0.056	NS
Total duration of locomotion (min/30min)	7.47 ± 0.92	5.80 ± 1.01	0.240	NS
Number of locomotory bouts (No/30min)	4.40 ± 0.40	2.60 ± 0.47	0.009	SIGN
Length of a locomotory bout (min/30min)	1.81 ± 0.26	2.49 ± 0.63	0.341	NS
Total duration of rest (min/30min)	0.93 ± 0.55	3.40 ± 1.50	0.141	NS
Number of rest bouts (No/30min)	0.60 ± 0.40	0.90 ± 0.40	0.605	NS
Length of a rest bout (min/30min)	0.53 ± 0.27	1.90 ± 0.66	0.072	NS

n=10 for each parameter

Table 11

Feeding responses of the first instar *M.testulalis* larvae on excised and intact (attached) common bean flowers

Parameter measured	Plant part offered: Excised Mean ± S.E	Attached Mean ± S.E	T	P< 0.05
Total duration of feeding (min/30min)	17.65 ± 1.47	19.00 ± 1.49	0.530	NS
Number of feeding bouts (No/30min)	4.20 ± 0.48	4.40 ± 0.33	0.692	NS
Length of a feed bout (min/30min)	6.25 ± 2.36	4.53 ± 0.56	0.510	NS
Total duration of locomotion (min/30min)	9.25 ± 1.70	10.55 ± 1.65	0.592	NS
Number of locomotory bouts (No/30min)	3.40 ± 0.71	3.66 ± 0.47	0.765	NS
Length of a locomotory bout (min/30min)	2.95 ± 0.24	2.82 ± 0.49	0.814	NS
Total duration of rest (min/30min)	3.25 ± 1.52	0.44 ± 0.44	0.110	NS
Number of rest bouts (no/30min)	1.00 ± 0.47	0.11 ± 0.11	0.090	NS
Length of a rest bout (min/30min)	1.47 ± 0.69	0.11 ± 0.11	0.080	NS

n=10 for each parameter

4.3.4 Feeding responses of first instar *M. testulalis* on cotton leaf and flower

Larvae fed for a significantly ($p < 0.05$) longer duration on the cotton flower than on the leaf. Feeding frequency and duration were however similar on both parts (Table 12). There were no significant differences ($P < 0.05$) in the total duration of locomotion, frequency and length of a locomotory bout on both parts of the plant. Rest duration was however, significantly higher on the leaf than on the flower.

4.3.5 Comparison of feeding patterns between first and fourth instar *M.testulalis* larvae on cowpea.

First instar larvae showed different feeding patterns from those of fourth instar larvae particularly with respect to the part of the plant presented. On leaves and flowers, duration and frequency of feeding were significantly higher in the first instar than in the fourth instar larvae but locomotor and rest activity in fourth instar larvae were significantly higher than in the first instar larva (Tables 13 and 14). In the latter, larval rest duration accounted for the longest time on cowpea leaf. On pods, locomotor activity was significantly longer in fourth instar than in the first instar (Table 15).

Table 12

Feeding responses of first instar *M.testulalis* larvae on cotton leaves and flowers

Parameter	Part	
	Leaf	Flowers
Total duration of feeding (min/0.5hr)	17.0 ± 1.25a	20.5 ± 0.79b
Frequency of feeding (No/0.5hr)	4.11 ± 0.28a	4.20 ± 0.24a
Average duration of individual feeding bouts (min)	4.90 ± 0.63a	5.24 ± 0.41a
Total duration of movements in between feeding bouts (min/0.5hr)	8.41 ± 1.12a	8.57 ± 0.92 a
Frequency of movement (No/0.5hr)	3.47 ± 0.40a	3.26 ± 0.32a
Average duration of individual movement (min)	2.44 ± 0.22a	2.86 ± 0.35a
Resting period in between feeding bouts (min/0.5hr)	4.64 ± 1.34a	0.94 ± 0.52b
Resting frequency (No/0.5hr)	1.53 ± 0.41a	0.36 ± 0.20b
Average duration of individual rests (min)	1.54 ± 0.36a	0.38 ± 0.21b

Means followed by the same letter in the same row for each plant part are not significantly different at p=0.05

Table 13

Feeding responses of first and fourth instar *M. testulalis* larvae on cowpea leaf.

Parameter	1st instar	4th instar
TDF	16.7 ± 1.22a	4.65 ± 0.81b
NFB	4.80 ± 0.29a	2.70 ± 0.40b
ADFB	3.64 ± 0.48a	1.68 ± 0.18b
TDM	6.95 ± 1.29b	12.1 ± 1.56a
NMB	3.50 ± 0.48a	3.90 ± 0.34a
ADMB	2.02 ± 0.21b	3.21 ± 0.38a
TDR	6.44 ± 2.02b	13.3 ± 2.22a
NRB	2.30 ± 0.70a	3.10 ± 0.48a
ADRB	1.70 ± 0.47b	4.19 ± 0.47a

Means followed by the same letter in the same row are not significantly different (p=0.05)

KEY
TDF=Total duration of feeding (min/0.5hr)
NFB=Number of feeding bouts (no/0.5hr)
ADFB=Average duration of individual feed bout (min)
TDM=Total duration of movement (min/0.5hr)
NMB=Number of movement bouts (no/0.5hr)
ADMB=Average duration of individual movement bout (min)
TDR=Total duration of rest (min/0.5hr)
NRB=Number of rest bouts (No/0.5hr)
ADRB=Average duration of each rest bout (min)

Table 14

Feeding responses of first and fourth instar *M. testulalis* larvae on cowpea flower.

Parameter	1st instar	4th instar
TDF	20.4 ± 1.00a	12.4 ± 0.73b
NFB	5.40 ± 0.45a	6.40 ± 0.34a
ADFB	4.16 ± 0.54a	1.90 ± 0.08b
TDM	9.09 ± 1.17b	14.0 ± 0.65a
NMB	4.60 ± 0.56b	6.60 ± 0.30a
ADMB	2.09 ± 0.21a	2.15 ± 0.11a
TDR	0.50 ± 0.27b	3.65 ± 0.95a
NRB	0.30 ± 0.15b	1.90 ± 0.48a
ADRB	0.50 ± 0.26b	1.46 ± 0.27a

Means followed by the same letter in the same row are not significantly different (p=0.05)

KEY
TDF=Total duration of feeding (min/0.5hr)
NFB=Number of feeding bouts (no/0.5hr)
ADFB=Average duration of individual feed bout (min)
TDM=Total duration of movement (min/0.5hr)
NMB=Number of movement bouts (no/0.5hr)
ADMB=Average duration of individual movement bout (min)
TDR=Total duration of rest (min/0.5hr)
NRB=Number of rest bouts (No/0.5hr)
ADRB=Average duration of each rest bout (min)

Table 15

Feeding responses of first and fourth instar *M. testulalis* larvae on cowpea pod.

Parameter	1st instar	4th instar
TDF	21.9 ± 1.05a	20.7 ± 0.56a
NFB	5.00 ± 0.25a	5.00 ± 0.21a
ADFB	4.56 ± 0.39a	4.21 ± 0.20a
TDM	6.70 ± 0.67b	9.00 ± 0.55a
NMB	3.90 ± 0.23a	4.30 ± 0.26a
ADMB	1.74 ± 0.15a	2.19 ± 0.21a
TDR	1.35 ± 0.76a	0.30 ± 0.21a
NRB	0.80 ± 0.42a	0.30 ± 0.21a
ADRB	0.64 ± 0.27a	0.20 ± 0.13a

Means followed by the same letter in the same row are not significantly (p=0.05)

KEY
TDF=Total duration of feeding (min/0.5hr)
NFB=Number of feeding bouts (no/0.5hr)
ADFB=Average duration of individual feed bout (min)
TDM=Total duration of movement (min/0.5hr)
NMB=Number of movement bouts (no/0.5hr)
ADMB=Average duration of individual movement bout (min)
TDR=Total duration of rest (min/0.5hr)
NRB=Number of rest bouts (No/0.5hr)
ADRB=Average duration of each rest bout (min)

4.3.6 The effect of prior feeding and food deprivation on feeding responses of first instar *M. testulalis* larvae

There was no variation in the time spent on feeding on cowpea leaves and flowers (Tables 16 and 17) at the 4 successive time intervals among larvae that were exposed to food prior the experiment. However, the frequency and duration of feeding bouts were highest during the first 30 minutes following onset of feeding and lowest at the 91-120 minute interval on both parts of the plant. The time spent on locomotor activity and frequency of locomotion were highest during the first 30 minutes while the shortest were observed during the 91-120 min. However, the duration of each movement bout remained fairly constant over the 2 hour period. There was an increase in the total duration and frequency of rest over the 2 hour period. The shortest rest durations were observed during the first 30 minutes and the longest durations during the 91-120 minutes.

On cowpea flower, larvae starved for 4 hours fed significantly longer than larvae that were not starved or starved for less than 1 hour. Frequency of feeding was higher in larvae starved for 4 hours than those starved for 3 hours or less (Table 18). On cowpea pod, starvation of larvae up to 4 hours neither affected

durations of feeding or rest activities. Locomotor activity was exceptional. Larvae starved for 4 hours appeared to be in locomotion for longer bouts than those starved for 1 hour but the differences were not statistically significant (Table 19).

Table 16

Effects of previous feeding on subsequent feeding responses of the first instar *M. testulalis* larva on cowpea leaves

Parameter	Time interval 0-30min	31-60min	61-90min	91-120min
Total duration of feeding (min/0.5hr)	16.1 ± 1.31a	15.4 ± 0.92a	15.7 ± 1.18a	14.50 ± 1.19a
Frequency of feeding (No/0.5hr)	5.00 ± 0.33a	4.50 ± 0.42ba	3.60 ± 0.28b	3.80 ± 0.20b
Average duration of individual feeding bouts (min)	3.42 ± 0.43a	3.63 ± 0.31a	4.86 ± 0.84a	3.86 ± 0.29a
Total duration of movements in between feeding bouts (min/0.5hr)	10.9 ± 0.85a	8.50 ± 0.94a	7.50 ± 0.87b	6.10 ± 0.68b
Frequency of movement (No/0.5hr)	4.70 ± 0.33a	3.70 ± 0.33ba	3.30 ± 0.43bc	2.50 ± 0.34c
Average duration of individual movement (min)	2.39 ± 0.19a	2.35 ± 0.26a	2.45 ± 0.26a	2.57 ± 0.28a
Resting period in between feeding (min/0.5hr)	2.95 ± 0.96c	6.10 ± 1.06b	6.77 ± 1.50ba	9.40 ± 1.27a
Resting frequency (No/0.5hr)	1.30 ± 0.42b	2.60 ± 0.33a	2.27 ± 0.38ba	3.10 ± 0.37a
Average duration of individual rests (min)	1.35 ± 0.39b	2.34 ± 0.22ab	3.11 ± 0.68a	2.77 ± 0.39a

Means followed by the same letter in the same row are not significantly different at $p < 0.05$

Table 17

Effect of previous feeding on subsequent feeding patterns of the first instar *M. testulalis* larva on cowpea flowers

Parameter	Time interval 0-30min	31-60min	61-90min	91-120min
Total duration of feeding (min/ 0.5hr)	22.15 ± 1.17a	23.1 ± 0.67a	22.1 ± 0.74a	23.1 ± 0.35a
Frequency of feeding (No/0.5hr)	5.30 ± 0.33a	4.20 ± 0.42b	3.90 ± 0.31b	4.10 ± 0.35b
Average duration of individual feeding bouts (min)	4.39 ± 0.38a	6.23 ± 0.89a	6.02 ± 0.51a	6.04 ± 0.54a
Total duration of movements in between feeding bouts(min/0.5hr)	7.40 ± 1.22a	4.10 ± 0.76b	4.20 ± 0.80b	3.75 ± 0.48b
Frequency of movement (No/0.5hr)	4.40 ± 0.49a	2.30 ± 0.37b	2.50 ± 0.34b	2.40 ± 0.34b
Average duration of individual movement (min)	1.67 ± 0.12a	1.78 ± 0.09a	1.44 ± 0.20a	1.66 ± 0.14a
Resting period in between feeding (min/0.5hr)	0.45 ± 0.24b	2.80 ± 0.82a	3.70 ± 0.42a	2.90 ± 0.49a
Resting frequency (No/0.5hr)	0.30 ± 0.15b	1.60 ± 0.37a	2.20 ± 0.20a	1.80 ± 0.33a
Average duration of individual rests (min)	0.45 ± 0.24b	1.18 ± 0.30ab	1.76 ± 0.20a	1.60 ± 0.30a

Means followed by the same letter in the same row are not significantly different at p = 0.05

Table 18

Effect of starvation on the feeding patterns of first instar *M. testulalis* larvae on cowpea flowers.

HRS OF STARVATION	Behavioural parameters examined								
	TDF	NFB	ADFB	TDM	NMB	ADMB	TDR	NRB	ADRB
0	18.75 ± 0.97C	4.58 ± 0.37AB	4.42 ± 0.35BC	6.41 ± 0.76B	3.50 ± 0.41AB	1.89 ± 0.18B	5.00 ± 1.29A	1.75 ± 0.44A	2.35 ± 0.64A
1	19.50 ± 0.28BC	5.08 ± 0.14A	3.87 ± 0.12C	9.00 ± 0.43A	4.33 ± 0.33A	2.29 ± 0.17A	1.50 ± 0.43B	0.91 ± 0.28A	1.02 ± 0.29B
2	21.83 ± 0.48A	4.33 ± 0.18B	5.17 ± 1.08BC	7.54 ± 0.53AB	3.50 ± 0.19B	2.19 ± 0.11A	0.63 ± 0.36B	0.33 ± 0.18B	0.48 ± 0.28B
3	20.88 ± 0.45AB	4.75 ± 0.21AB	4.51 ± 0.25BC	7.29 ± 0.49B	3.42 ± 0.19B	2.13 ± 0.09A	1.83 ± 0.58B	1.25 ± 0.37BA	0.93 ± 0.21B
4	22.58 ± 0.54A	4.25 ± 0.18B	5.46 ± 0.35A	6.58 ± 0.43B	3.41 ± 0.23B	1.96 ± 0.12A	0.83 ± 0.29B	0.67 ± 0.22B	0.63 ± 0.21B

Means followed by the same letter in the same column are not statistically significant at $P < 0.05$ (D.M.R.T)

KEY
TDF=Total duration of feeding (min/0.5hr)
NFB=Number of feeding bouts (no/0.5hr)
ADFB=Average duration of individual feed bout (min)
TDM=Total duration of movement (min/0.5hr)
NMB=Number of movement bouts (no/0.5hr)
ADMB=Average duration of individual movement bout (min)
TDR=Total duration of rest (min/0.5hr)
NRB=Number of rest bouts (No/0.5hr)
ADRB=Average duration of each rest bout (min)

Table 19

Effect of starvation on the feeding patterns of first instar *M. testulalis* larvae on cowpea pod

Hrs of starvation	Behavioural parameters examined								
	TDF	NFB	ADFB	TDM	NMB	ADMB	TDR	NRB	ADRB
0	21.79 ± 0.88A	5.08 ± 0.22A	4.43 ± 0.33A	6.75 ± 0.56A	3.75 ± 0.22A	1.83 ± 0.15B	1.38 ± 0.69A	0.67 ± 0.38A	0.70 ± 0.26A
1	19.71 ± 0.52A	4.83 ± 0.24A	4.25 ± 0.33AB	9.38 ± 0.56A	4.17 ± 0.24A	2.28 ± 0.11AB	0.92 ± 0.31A	0.50 ± 0.19A	0.82 ± 0.27A
2	21.33 ± 0.59A	4.58 ± 0.15A	4.48 ± 0.18A	8.58 ± 0.53AB	4.00 ± 0.21A	2.19 ± 0.15AB	1.08 ± 0.44A	0.58 ± 0.23A	0.78 ± 0.29A
3	20.21 ± 0.51A	5.00 ± 0.21A	4.13 ± 0.20A	9.29 ± 0.50A	4.33 ± 0.18A	2.17 ± 0.11AB	0.50 ± 0.28A	0.33 ± 0.18A	0.37 ± 0.20A
4	20.30 ± 0.90A	4.50 ± 0.26A	4.75 ± 0.38A	9.08 ± 1.05A	3.83 ± 0.21A	2.36 ± 0.23A	0.58 ± 0.23A	0.33 ± 0.14A	0.58 ± 0.26A

Means followed by the same letter in the same column are not statistically significant at $P < 0.05$ (D.M.R.T)

KEY
TDF = Total duration of feeding (min/0.5hr)
NFB = Number of feeding bouts (no/0.5hr)
ADFB = Average duration of individual feed bout (min)
TDM = Total duration of movement (min/0.5hr)
NMB = Number of movement bouts (no/0.5hr)
ADMB = Average duration of individual movement bout (min)
TDR = Total duration of rest (min/0.5hr)
NRB = Number of rest bouts (No/0.5hr)
ADRB = Average duration of each rest bout (min)

4.4 Discussion

4.4.1 Feeding responses of the first instar *M. testulalis* larvae with respect to time of the day

Feeding activity of the first instar *M. testulalis* larvae on host plant parts consists of feeding bouts alternating with non-feeding periods of rest and or movement on the substrate. This behavioural pattern has been reported in other lepidopteran larvae (Ma, 1972; Bernays and Simpson 1982).

The duration and frequency of individual feeding bouts on host leaves and flowers by the first instar larvae did not seem to vary with the time of day (Table 4). Other activity patterns in between feeding bouts such as locomotion and rests were also not influenced by the time of day (Tables 5 and 6). Similarly, diel cycle had no influence on the quantity of food intake on different parts of cowpea except on stems where larvae ate significantly more material during the day than at night. On common beans, there was no clear diel influence on feeding because, whereas larvae ate significantly more pod and seed material at night, they ate more stem during the day. Clearly, if feeding was under the influence of the

diel cycle we would have expected more feeding either at night or during the day. It appears that differences in feeding observed are due to factors other than light regime. For example, cowpea flowers evoked longer feed bouts than leaves thus suggesting that the larvae prefer to feed on flowers. Furthermore, intervals between feeding bouts were longer on cowpea leaves than on flowers. This suggests that feeding on the leaves is sporadic and this is possibly due to lack of adequate phagostimulants in the cowpea leaves. It was also observed that locomotion activity on the cowpea and common bean flowers was higher than that on the cowpea leaf. It appears that the increased locomotor activity in cowpea and *Phaseolus* flowers is associated with the longer feeding duration observed in these parts.

Some workers have alluded to the nocturnal habits of the adult *M. testulalis* especially with respect to oviposition (Okeyo Owour and Ochieng, 1981) and they have even suggested that it is a nocturnal insect. The results obtained here appear to suggest that feeding in the larval stage of the insect is not under any diel influence. Circadian induced rhythms in feeding are well known in blood sucking diptera as well as in some lepidopterous larvae (Bernays and Simpson, 1982) but according to Beck (1980), only a few lepidopterous

larvae show these rhythms because most of them are relatively sessile compared to host seeking mosquitoes and other blood sucking diptera whose feeding patterns are known to be regulated by changes in the photoperiod. From the available data it seems, therefore, that the photoperiod appears to have no influence on the feeding pattern of the first instar *M. testulalis* larva on its host plants.

4.4.2 Feeding responses of the first and fourth instar larvae on the host plant parts

The shorter duration of feeding on cowpea leaf in the fourth instar larvae suggests that cowpea leaf is less palatable than the flower or pod. This suggestion is collaborated by total duration of rests which was significantly higher in the fourth instar than in the first instar as shown clearly in Table 13. It is most likely that, the fourth instar larvae rested more so as to enable digestion of the leaf. This does not happen with the first instar larvae because the leaves are natural foods which larvae feed on as soon as they hatch. The fourth instar larvae also moved about significantly more on cowpea leaf than the first instar larva. This behaviour could be related to the searching activity aimed at finding a more palatable and suitable source of food.

Feeding pattern of the first and fourth instar larvae was similar on cowpea pod. This is probably due to the high palatability of the pods compared to the flowers and leaves. The longer locomotory periods of the fourth instar larvae on the pods may be related to the behaviour of the larva on highly palatable food materials.

4.4.3 Feeding responses on intact and excised parts

Longer durations of feeding on intact cowpea leaves suggest that the feeding pattern of larvae is to a certain extent influenced by the state of the plant or biochemical components of the food. First instar *M. testulalis* larvae appeared to prefer feeding on attached leaves as opposed to the excised leaf. Excision of the leaf appeared to reduce the feeding period. This implies that some biochemical changes could have occurred in the excised cowpea leaf which were responsible for the decreased feeding. Feeding responses by the larvae on cowpea and common bean flowers were independent of whether these parts were intact or excised. This observation indicates that excision of flowers may have no adverse effect on feeding by *Maruca* larvae and implies that the larvae are capable of feeding on the flowers even after they have been shed from the parent plant. It is however

important to bear in mind that many workers have shown that sometimes one cannot equate feeding in the laboratory with that in the field (Raguse and Smith 1965; McGinnis and Kastings, 1966; Harley and Thorsteinson, 1967; Bernays and Chapman, 1975, Bernays *et al.* 1977; McCaferry, 1982). Results from this study indicate that some biochemical changes occur in excised cowpea leaves that inhibit feeding. Such changes may not be readily occurring in the flowers of both host plants and if they do the changes that take place in the flower may be so slow that normal feeding is not affected. This observation may be linked among other factors to poor feeding on cowpea leaf as opposed to cowpea flowers by later larval instars. It may also be linked to the level of nutrients and feeding stimuli in the flower compared with the leaves or to changes caused in the leaf due to desiccation.

4.4.4 Feeding responses of the first instar *M. testulalis* larvae on the non-host plant

The first instar larvae were able to feed on the cotton leaves and flowers in spite of its being a non-host. The larvae also spent longer durations of feeding on cotton flower than on the leaves. Furthermore, the rest activity was clearly longer on

the cotton leaf than on the flower while the duration of locomotor activity was higher on the flower than on the leaf. Cotton is a non-host plant for *M. testulalis* larva. The fact that newly emerged larvae are able to feed on it in a no-choice situation suggests that these parts of the non-host plant may not contain a feeding deterrent. It also suggests that larval insects are rather naive in their food selection behaviour. Cotton flowers evoked higher feeding compared to cotton leaves. This is an indication that they contain more phagostimulative compounds than the leaves. The longer rest activity on the leaves could be an indication that the larvae needed more time to digest the leaf material compared to the flower or the leaf lacks stimuli to sustain prolonged feeding activity. Cotton may thus be regarded as an acceptable non-host for the first instar *M. testulalis* larvae according to the classification of de Boer and Hanson (1984).

4.4.5 Influence of prior feeding and food deprivation on feeding patterns

Absence of significant variations in duration of feeding on cowpea leaf and flower at the four successive time intervals in unstarved larvae appears to suggest that the feeding pattern of *M. testulalis* is not similar to that known in locusts and

grasshoppers where a feeding bout is followed by a definite period of non-feeding and hence equated to a meal (Chapman and Beerling, 1990; Simpson, 1990). In *M. testulalis* a feeding bout was followed by rest or movement which lasted very brief durations suggesting that these events were merely part of the feeding repertoire rather than an indication that the insect was satiated. The long locomotor activity during the first 60 minutes was most likely associated with establishment of a feeding site by the larvae on the plant substrate. However, the inverse relationship observed between rest and feeding durations appeared to suggest that the longer the larvae fed, the more they needed the rest probably to digest the consumed food.

Starvation of the larvae up to 4 hours prior feeding on cowpea flower resulted in longer durations and higher frequencies of feeding as opposed to larvae that were not starved. This suggests that there was a higher food uptake in starved larvae. This was however not true when the larvae were presented with the pods. This was probably due the fact that the pod is not natural food of the first instar larvae as opposed to flowers or leaves.

CHAPTER 5

QUANTITATIVE STUDIES OF LARVAL FEEDING OF FOURTH INSTAR *M. TESTULALIS* ON HOST AND NON-HOST PLANTS AND THEIR SUITABILITY FOR LARVAL GROWTH AND DEVELOPMENT

5.1 Food intake by fourth instar *M. testulalis* larvae on cowpea and common bean parts in a choice and no choice situation

5.1.1 Introduction

The fourth instar *Maruca testulalis* larvae feed mainly on the host plant pods as opposed to other parts. Experiments were carried out firstly to quantify feeding response of the fourth instar larvae on host and non-host plant parts in a no choice situation and secondly to find out using a choice test if the number of insects feeding on a particular plant part reflected the palatability observed in a no choice situation. The ultimate aim was to use the feeding responses as a measure of palatability of different parts of the host and non-host plants. The fourth instar stage was chosen because it is the larval instar which feeds most during the life cycle of *M. testulalis*.

Although several workers have reported differences in *M. testulalis* larval growth and development on susceptible and resistant cowpea cultivars (Jackai, 1981b; Mcfoy *et al.* 1983 and Okech, 1986), very little is known about *M. testulalis* larval growth and development on various host and non-host plants. Consequently, a study was undertaken to compare larval growth on cowpea and common bean (host plants) and cotton (non-host plant). Finally, the influence of larval food on the feeding preferences of the penultimate larval instars of *M. testulalis* was investigated to find out whether the larvae retain a normal host selection behaviour when reared on one host and then offered another host to feed on.

5.2 Materials and Methods

5.2.1 Food intake in choice and no choice situations

Feeding materials used were fresh excised leaves, stems, flowers, developing pods and seeds from cowpea and common beans. Leaves and flowers from cotton were also tested. Each of the test material was placed in a plastic Petri dish (6 cm diameter) lined with moistened filter paper at the bottom. Each Petri dish was provided with one fourth instar larva previously

starved for 10 hours and left to feed for 12 hours. Forty larvae were used in each experiment. Measurement of food consumed during this period was calculated using the formula shown in section 4.2.2. Means were then compared with the Duncan's Multiple Range Test.

In the second experiment two choice tests were performed. In the first design, one cowpea part was paired with a corresponding part of the common bean and in the second design, different parts of cowpea (stem, flower, pod and seed) were paired with the cowpea leaf. Fourth instar larvae previously starved for 10 hours prior the experiment were then introduced into each Petri dish. The experiment lasted for 12 hours after which the parts in each Petri dish were inspected and the number of insects that fed on each part counted.

5.2.2 Suitability of different parts of cowpea, common beans and cotton for *M. testulalis* larval growth and development

Newly emerged *M. testulalis* larvae were reared separately on cowpea and common bean leaves, stem, flowers, pods and seeds and on cotton leaves, flowers and tender bolls in sandwich boxes measuring 10 x 16 x 5 cm (Plate 8). One half of the bottom part of box was lined with a wet pad of cotton to provide moisture for

the larvae. The remaining part was lined with a dry Kleenex paper tissue on which the food material was placed. Ten newly emerged larvae were introduced around the food using a fine camel hair brush. The boxes were then covered with a black cloth. Laboratory temperatures were not regulated and fluctuated between 26-30° C. Diet was changed every 2 days in the 1st and 2nd instars but replenished daily when the insects were in their 3rd, 4th or 5th instars until pupation. The following parameters were recorded from the experiment:

a) Developmental period from 1st instar larva to pupa.
c) Percent Pupation
d) Mean pupal weight

The experiment was replicated 3 times and an ANOVA was carried out using the general linear model to investigate the role of larval food on the above parameters.

5.2.3 Role of larval food on the feeding preferences of the fifth instar *M. testulalis* larvae

Newly emerged *M.testulalis* larvae were divided into two batches of 100 larvae each. One batch was reared exclusively on cowpea flowers and pods and the other on common bean flowers and pods. Rearing of first to fourth instars was done in plastic sandwich boxes similar to those used in previous experiment (see section 5.2.2). When the larvae in each batch had moulted into the fourth instar stage they were divided into 2 treatments: A,B, for those reared on cowpea and C,D for those reared on common bean as shown in the flow chart (Fig. 2). Thus, thirty larvae reared on cowpea, were offered cowpea pods to feed upon for 24 hours (Treatment A) which also acted as a control in this batch. The other half were offered bean pods to feed on for the same period of time (Treatment B). Larvae reared on beans were also divided into 2 groups. One group was offered cowpea pods (Treatment C) while the other was offered bean pods as a control for this batch (Treatment D). The pods were weighed before and after presentation to the larvae.

To get the dry weight per unit fresh weight of the pods, fresh cowpea and common bean pods were weighed and put in the oven to dry for 72 hours at 75° C. After the pods had dried thoroughly, they were again weighed to get their dry weights. The ratio of the fresh to dry weight was calculated as:

$$\text{Ratio: } \frac{\text{Dry weight}}{\text{Fresh weight}}.$$

To calculate the dry weights of the pods consumed, the fresh weight before feeding was subtracted from the fresh weight after feeding. The difference was then multiplied by the ratio obtained for each part. An anova was used to find out if rearing food causes induction of larval feeding preference.

5.3 Results

5.3.1 Feeding responses in no choice tests

Cowpea pods and flowers elicited highest feeding response (77.31 ± 4.02 mg and 75.00 ± 3.27 mg, respectively) in no choice tests (Table 20). Larvae fed on all parts of the inflorescence except the sepals. Feeding on pods occurred on the inner parts of the fleshy pericarp and ovules. Leaves and stem elicited significantly lower ($p < 0.05$) but similar feeding responses (64.00 ± 3.01 mg and 64.08 ± 4.42 mg

respectively) than flowers or pods. Larvae made punctures in the leaf blade as they fed but veins were avoided. On stems, only the soft parenchymatous tissues of the pith were consumed. Developing seeds evoked the least feeding response (51.73 ± 2.43 mg) which was significantly different from all the others (Table 20).

Feeding response on common bean leaves was very low because all larvae got trapped in the trichomes which prevented locomotion and hampered feeding resulting in very little feeding (1.00 ± 0.20 mg). Some larvae were pierced by the trichomes causing 60 % mortality among experimental insects. Common bean pods and flowers elicited very high feeding responses (136.07 ± 10.72 mg and 133.13 ± 57.06 mg, respectively) followed by stems (67.97 ± 4.00 mg) while on seeds, 55.61 ± 2.91 mg was consumed (Table 20).

Larvae did not feed on cotton leaves and flowers and therefore no measurements of feeding responses were obtained.

5.3.2 Feeding responses in choice tests

Larvae clearly choose what part of host plant to feed on (Table 21). Sixty percent of the larvae fed on cowpea seeds while 40 % fed on common bean seeds. With the pods, a majority (57 %) fed on common bean and 43 % fed on cowpea. None of the larvae in the above choice tests fed on both parts. In the choice test involving flowers, 54 % preferred common bean and 40 % fed on cowpea while 6 % fed on both parts. The choice test with stems had the highest percentage (23 %) of larvae that fed on both parts. However, the majority preferred cowpea stem (45 %) as opposed to 21 % on common beans. Eleven percent of the insects here did not choose any of the two parts for feeding. In a choice between host plant leaves, 53 % of the larvae fed on cowpea, 41 % were found trapped on the bean leaf and 6 % fed on both leaves. (Table 21).

In the second choice experiment in which the percentage numbers of larvae feeding on different parts of cowpea were compared against those that chose cowpea leaf, more larvae were recorded on stem, flower, pod or seed in preference to the leaf. A preference index showed the following diminishing order of preference for the different parts: pod > flower > stem > seed > leaf (Table 22).

Table 20

Food intake by fourth instar *M. testulalis* on different parts of cowpea and common bean under no choice

Quantity of food (mg fresh weight) ingested in 12 hours			
Host plant part	Cowpea Mean ± S.E	Common bean Mean ± S.E	Cotton Mean ± S.E
Leaves	64.00 ± 3.01Ba	1.00 ± 0.20Db	0
Stem	64.08 ± 4.42Ba	67.97 ± 4.00BCa	0
Flowers	75.00 ± 3.27Aa	133.13 ± 57.06Ba	0
Pods	77.31 ± 4.20Ab	136.07 ± 10.72Aa	*
Seeds	51.73 ± 2.41Ca	55.61 ± 2.91Ca	*

1. Means with the same capital letter in the same column or with the same small letter in the same row are not significantly different at P=0.05.

2. Duncan groupings are based on square root transformed data.

* Parts that were not tested

used in the calculations of water loss. Weight of food ingested was calculated through the following steps:

Initial plant part weight= D1

Final plant part weight= D2

Initial plant part weight (control)= C1

Final plant part weight (control)= C2

Weight loss due to evaporation= (C1-C2)

Rate of weight loss per initial weight of plant part in the control= C1-C2/C1

Calculated weight loss of initial plant part= C1-C2/C1xD1

Weight of ingested food when water loss is compensated= D1-(D2+(C1-C2/C1xD1)).

Forty larvae were used in each experiment and data obtained were analysed by the general linear model (G.L.M). Means were separated with Duncan Multiple Range Test.

4.3 Results

4.3.1 Feeding responses of the first instar *M. testulalis* larvae with respect to time of the day

M.testulalis larva fed individually at a site which they guarded from other larvae. Feeding surpassed locomotor and rest activities on host plant parts. After each feeding bout, larvae either moved away or rested before resuming feeding at the old spot. During these activities the larvae paused briefly to defeacate.

Larvae spent longer periods feeding on cowpea flowers than on leaves (Table 4). The frequency of feeding on the leaf material was similar to that observed on the flower material but feeding bouts were longer on flower than on leaf material. The total time spent feeding, frequency of feeding and duration of each feeding bout were, however, similar at different times of the photoperiod on both parts of the cowpea. On common bean flower tissue, larval feeding responses appeared to be time dependent. Larvae fed for longer periods between 20.00-24.00 hours than between 8.00-12.00 hours (Table 4).

On cowpea flower, the first instar larvae spent more time on locomotor activity than on leaves. The frequency of movement was correspondingly higher on flowers than leaves but the duration of each locomotor bout was similar on both parts. Time of day had no effect on time spent on locomotory activity, its frequency and duration on both parts of cowpea. A similar locomotor trend was observed on common bean flowers although duration of locomotion bouts at 14.00-18.00 hours were lower than that between 8.00-12.00 hours (Table 5).

Larva rested significantly longer ($p < 0.05$) on cowpea flower than on leaves. Similarly, the frequency of rests and duration of rest bouts were higher on the leaf than on the flowers. These larval behaviours were however unaffected by changes in the time of day (Table 6). Similar results were obtained on common bean flowers although the larvae appeared to rest for a shorter period at 20-24 hrs (Table 6) than at all the other periods investigated.

Table 4

Feeding pattern of first instar *M. testulalis* larvae on host plant leaves and flowers during different times of the day

Plant part	Parameter	Time of day (hours) 8.00-12.00	14.00-18.00	20.00-24.00
Cowpea leaf				
	Total duration of feeding (min/0.5hr)	16.7 ± 1.44a	16.10 ± 1.47a	16.20 ± 1.08a
	Frequency of feeding (No/0.5hr)	4.80 ± 0.3a	4.90 ± 0.3a	5.70 ± 0.5a
	Average duration of individual feeding bouts (min)	3.66 ± 0.47a	3.40 ± 0.38a	2.90 ± 0.28a
Cowpea flowers				
	Total duration of feeding (min/0.5hr)	21.6 ± 0.76a	21.6 ± 1.06a	22.4 ± 1.62a
	Frequency of feeding (No/0.5hr)	5.30 ± 0.36a	4.80 ± 0.44a	0.75 ± 0.63a
	Average duration of individual feeding bouts (min)	4.35 ± 0.47a	5.22 ± 1.02a	0.20 ± 0.47a
Common bean flowers				
	Total duration of feeding (min/0.5hr)	17.6 ± 1.47b	20.6 ± 1.03ab	22.8 ± 1.88a
	Frequency of feeding (No/0.5hr)	4.20 ± 0.48a	4.80 ± 0.39a	3.50 ± 0.28a
	Average duration of individual feeding bouts (min)	6.25 ± 2.36a	4.40 ± 0.32a	6.73 ± 1.08a

Means followed by the same letter in the same row are not significantly different at p=0.05

Table 5

Variations in locomotory patterns of first instar *M. testulalis* larvae on some of its host plant parts with time of day.

Plant part	Parameter	Time of day (hours) 8.00-12.00	14.00-18.00	20.00-24.00
Cowpea leaf				
	Total duration of movements in between feeding bouts (min/0.5hr)	5.23 ± 1.17a	6.83 ± 1.54a	7.65 ± 1.77a
	Frequency of movement (No/0.5hr)	2.60 ± 0.42a	3.13 ± 0.61a	3.33 ± 0.66a
	Average duration of individual movement (min)	2.15 ± 0.33a	2.29 ± 0.54a	2.24 ± 0.57a
Cowpea flower				
	Total duration of movements in between feeding bouts (min/0.5hr)	7.47 ± 0.92a	6.34 ± 0.88a	5.27 ± 1.07a
	Frequency of movement (No/0.5hr)	4.40 ± 0.40a	3.10 ± 0.43a	4.00 ± 0.81a
	Average duration of individual movement (min)	1.81 ± 0.26a	2.14 ± 0.18a	1.55 ± 0.51a
Common bean flowers				
	Total duration of movements during feeding (min/0.5hr)	1.25 ± 1.70a	7.12 ± 1.82a	6.75 ± 1.60a
	Frequency of movement (No/0.5hr)	3.40 ± 0.71a	3.50 ± 0.77a	3.25 ± 0.47a
	Average duration of individual movement (min)	2.95 ± 0.24a	1.71 ± 0.39b	2.01 ± 0.25ab

Means followed by the same letter in the same column are not statistically significant at p=0.05

Table 6

Variations in the rest patterns of first instar *M. testulalis* on some of its host plant parts with time of day.

Plant part	Parameter	Time of day (hours) 8.00-12.00	14.00-18.00	20.00-24.00
Cowpea leaf				
	Resting period in between feeding bouts (min/0.5hr)	8.12 ± 1.73a	7.00 ± 1.34a	6.76 ± 1.55a
	Resting frequency (No/0.5hr)	2.90 ± 0.58a	2.50 ± 0.46a	2.88 ± 0.45a
	Average duration of individual rests (min)	2.23 ± 0.41a	2.48 ± 0.43a	2.23 ± 0.38a
Cowpea flower				
	Resting period in between feeding bouts (min/0.5hr)	0.93 ± 0.55a	2.15 ± 1.00a	1.75 ± 1.75a
	Resting frequency (No/0.5hr)	0.60 ± 0.40a	1.40 ± 0.54a	0.25 + 0.25a
	Average duration of individual rests (min)	0.53 ± 0.28a	0.76 ± 0.29a	1.75 ± 1.75a
Common bean flowers				
	Resting period in between feeding duration (min/0.5hr)	3.25 ± 1.52a	3.06 ± 0.96a	0.50 ± 0.50a
	Resting frequency (No/0.5hr)	1.00 ± 0.47ab	2.00 ± 0.46a	0.25 ± 0.25b
	Average duration of individual rests (min)	1.48 ± 0.69a	1.36 ± 0.21a	0.50 ± 0.50a

Means followed by the same letter in the same row for each plant part are not significantly different at p=0.05

4.3.2 Food intake in fourth instar *M. testulalis* larvae in relation to time of day

There was no significant difference in the quantity of food intake by the fourth instar *M. testulalis* larvae during day or night on cowpea leaf, flower, pod and seeds. However, larvae fed significantly more on cowpea stem during day than at night (Table 7). On common beans, the quantities of flower and leaf eaten during day or night were not significantly different. However, as in cowpea, larvae ate significantly more stem during day than at night. Consumption of pods and seeds was significantly more at night than during day (Table 8).

Table 7

Influence of time of day on intake of different parts of cowpea by fourth instar *M. testulalis* larvae.

Quantity of food (mg fresh weight) ingested in 12 hours		
Cowpea part	Day feeding Mean ± S.E	Night feeding Mean ± S.E
Leaves	64.94 ± 4.16a	62.88 ± 4.39a
Stem	83.26 ± 7.26a	45.35 ± 3.11b
Flowers	66.02 ± 3.16a	84.90 ± 5.44a
Pods	85.15 ± 4.58a	68.10 ± 7.17a
Seeds	42.40 ± 1.72a	60.82 ± 4.05a

Means followed by the same letter in the same row are not significantly different at p=0.05.

Table 8

Influence of time of day on intake of different parts of common bean by fourth instar *M. testulalis* larvae.

Quantity of food (mg fresh weight) ingested in 12 hours

Cowpea part	Day feeding Mean ± S.E	Night feeding Mean ± S.E
Leaves	6.78 ± 2.12a	3.14 ± 1.25a
Stem	83.82 ± 6.08a	52.52 ± 3.93b
Flowers	70.84 ± 4.74a	79.72 ± 7.18a
Pods	91.06 ± 8.63a	177.54 ± 16.51b
Seeds	42.46 ± 3.69a	64.17 ± 3.65b

Means followed by the same letter in the same row are not significantly different at p=0.05.

4.3.3 Feeding responses of first instar *M. testulalis* with respect to condition of the plants

4.3.3.1 Responses on cowpea leaves

The larvae spent a significantly longer time on feeding on intact cowpea leaves as opposed to excised leaves ($t=0.07$, $p < 0.05$). The frequency of feeding and duration of each feeding bout on excised leaves were however not significantly different from those of intact leaves (Table 9). Time spent on locomotion and duration of each locomotory bout in between feeding periods on both leaf types were similar but the frequency of locomotion on the attached leaves was higher than that on the excised leaves. Larval rests were longer (8.12 min) on excised than on intact leaves (1.15 min) (Table 9).

4.3.3.2 Responses on cowpea flowers

On cowpea flowers there were no significant differences in the total time spent on feeding and duration of feeding bouts by the larvae between the excised and intact flowers (Table 10). However, the frequency of larval feeding on excised flowers was higher than that on attached flowers. Locomotory and rest patterns were not different between excised and intact parts. (Table 10).

4.3.3.3 Responses on common bean flowers

On common bean flowers there were no significant differences in the pattern of feeding by first instar larvae between excised and intact flowers for all the parameters measured (Table 11).

Plate 10: Scanning electron micrographs of the fifth instar *M. testulalis* larva mouth parts:

(A) Maxillary galea showing palpal appendage (Pa) bearing tip (t) with sensilla; the 2 styloconica sensilla: lateral (Ls) and medial (Ms) and the spinneret (S).

(B) Higher magnification of the eight palpal tip sensilla (*).

(C) Lateral (Ls) and Medial (Ms) styloconica sensilla.

(D) Lateral styloconica sensilla (Ls) with terminal papillae (p) situated in a socket.

7.3 Results

7.3.1 Scanning electron microscope of the mouth part sensilla

The palp tip in *M. testulalis* stands on a robust appendage whose tip comprises 8 prominent sensilla (Plate 10). Five of these hairs have pores 0.5um wide. The galea bears 2 sensilla styloconica, medial one and a lateral one. The hair shaft of the lateral sensillum is shorter (ca. 20um) than the medial one (ca.40um) (Plate 10). The base diameter of the lateral sensillum is ca. 12.5um. The terminal papilla of both hairs are 3um long and open distally at the top. The terminal segment of the antennal tip has several hairs of which 3 are basiconica, one trichodea and at least 2 styloconica sensilla (Plate 11).

7.3.2 Feeding responses

7.3.2.1 Sugars

Discs impregnated with sucrose evoked better feeding response than those impregnated with glucose and the area ingested was directly proportional to the sugar concentration. The optimum concentration for sucrose was 0.2 M. while for glucose it was 0.05 M. (Fig. 5, Appendices 9, 10).

the glass micropipette with the help of a hypodermic syringe and slipped over the recording electrode.

7.2.4.3 Data recording and analysis

The tip cell recording method (Hodgson, 1955) was used. The recording system consisted of two preamplifiers connected in series. A Grass P16, fitted with Ag/AgCl microelectrode, was the primary amplifier. The indifferent electrode carried the glass micropipette bearing the larva head capsule, while the recording electrode carried the glass micropipette filled with the stimulating solution. Signals from the primary preamplifier were amplified again by a Grass P15 preamplifier and the output was simultaneously displayed on Tektronix 5A 18N dual trace Oscilloscope and simultaneously recorded on a Store 4DS Racal instrumentation tape recorder using Maxell XL 35-90B sound recording magnetic tape. Spikes generated during the first 1 sec of stimulus application were counted and means of impulses per second from 15 replicates were analysed using a regression analysis.

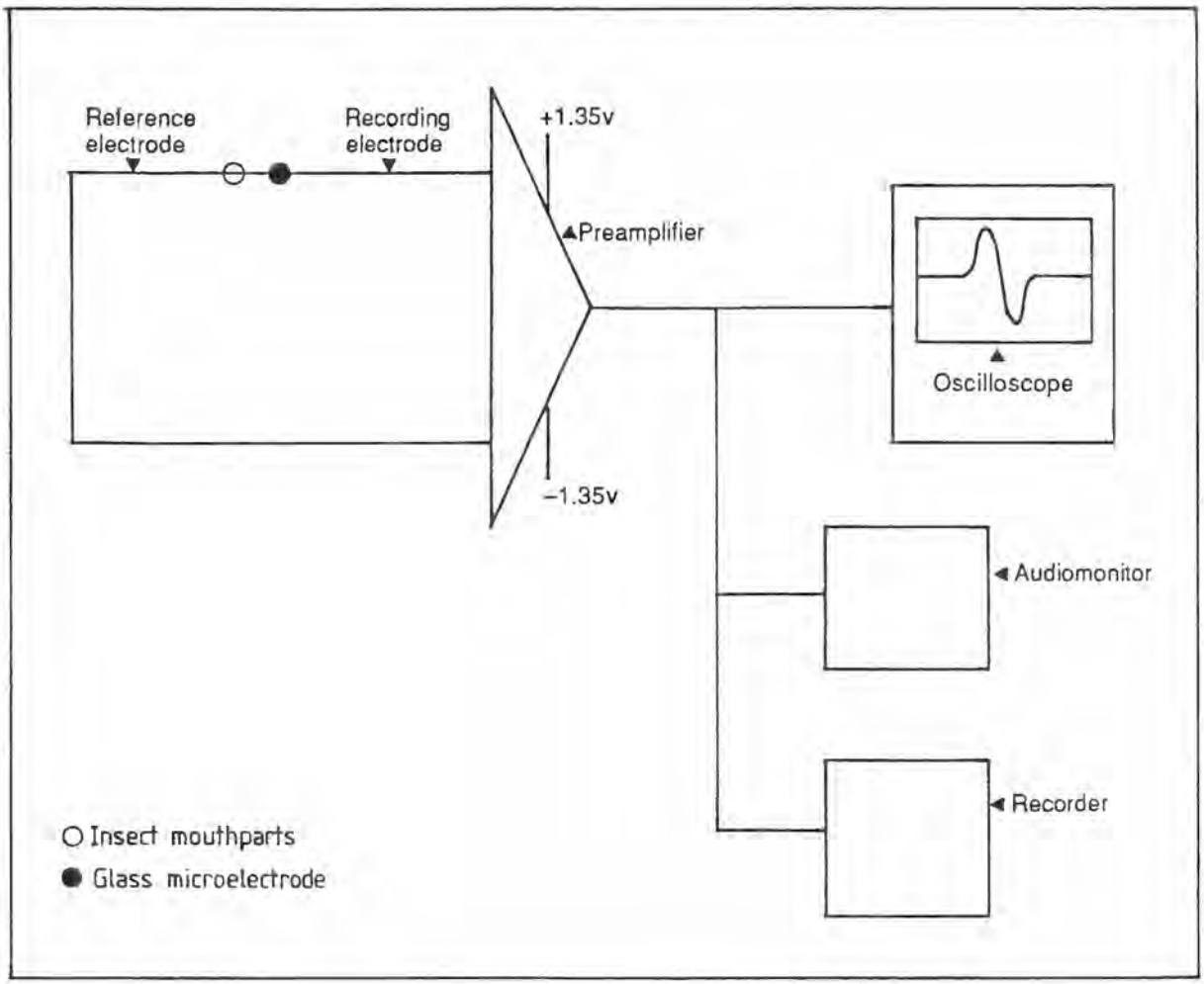


Figure 4: A diagrammatic representation of the electrical connections in the electrophysiological set up.

200 - 3.25 ug/ul. The stimulating solutions were stored in a deep freezer until required for use.

7.2.4.2 Preparation of the animal for recording

Fifth instar larvae previously starved for at least 2-3 hours prior the experiment were used in these studies. The larvae was decapitated at the junction of the 4th segment from the head. The posterior end of the head capsule was poked with a needle to destroy the brain thus eliminating extraneous neural and muscular activity. The head was then impaled on the shank of the glass micropipette filled with Beadle's saline prepared as described in section 3.4.4. This approach facilitated the extension of the required sensilla into the open thus making it easy to approach them with the recording electrode. The glass micropipette bearing preparation was gently slipped onto the reference electrode on the recording set up (see diagram in Fig. 4).

The tip of the glass micropipette to be fitted on the recording electrode was broken evenly under the dissecting microscope with the blunt end of the forceps to give a tip of approximately 5-10 um in diameter. The stimulating solution was introduced into

and minimised evaporation of water from the agar capsule throughout the experimental period. After 12 hours the volume of uneaten pellet was measured and subtracted from the original. Water loss from the agar was computed as detailed in section 4.2.2. A total of 30 larvae were used in each treatment. The experiment comprised 3 replicates with 10 insects per replicate and Anova was carried out using the general linear model to test the significance of feeding responses.

6.3 Results

6.3.1 Feeding responses on agar pellets containing host and non-host plant powders

Larvae did not feed on plain agar. However, the agar medium containing powder from cowpea and common bean seed material elicited significantly high quantities of larval feeding as opposed to that of the other parts of the host plants (Table 25). Agar gels containing powder from cotton parts evoked the least feeding responses and no feeding at all on the cotton flower.

6.3.2 Feeding responses of fifth instar *M. testulalis* larvae to sugars

Quantity of agar gel ingested by the larvae increased with the concentration of each of the 3 sugars sucrose, glucose and fructose till it became inhibitive after 0.2 M. (Table 26) Fructose elicited the least stimulatory effect at 0.01 M. (Table 26, Appendix 6) while sucrose evoked the highest feeding at the same concentration (Table 26, Appendix 7). Glucose stimulated more feeding than other sugars after 0.2M (Table 26, Appendix 8). Optimal concentration in all the 3 sugars was between 0.1- 0.2 M (Fig 3).

Table 25

Intake of cowpea, common bean and cotton powders incorporated separately in agar gel medium by fourth instar *M. testulalis* larvae

Volume of pellet (ml) ingested by 2 larvae in 12 hours			
	Host plant		
Plant powder	Cowpea Mean ± S.E	Common bean Mean ± S.E	Cotton Mean ± S.E
Leaves	0.15 ± 0.01B	0.09 ± 0.00B	0.03 ± 0.02A
Stem	0.12 ± 0.01BC	0.09 ± 0.01B	0.03 ± 0.03A
Flowers	0.12 ± 0.01BC	0.11 ± 0.01B	0.00 ± 0.00A
Pods/Bolls	0.11 ± 0.01C	0.09 ± 0.01B	0.03 ± 0.00A
Seeds	0.18 ± 0.01A	0.19 ± 0.01A	0.06 ± 0.03A

Means with the same capital letter in the same column are not significantly different at p=0.05.

Table 26

Intake of various sugars in agar-cellulose gel by fourth instar *M. testulalis*

	Mean weight (g) pellet consumed by 2 larvae in 24 hours		
Conc.	Sucrose (Mean ± S.E)	Glucose (Mean ± S.E)	Fructose (Mean ± S.E)
1 M	0.091 ± 0.013c	0.165 ± 0.018b	0.089 ± 0.007c
0.2 M	0.237 ± 0.017a	0.339 ± 0.021a	0.267 ± 0.023ba
0.1 M	0.216 ± 0.021a	0.180 ± 0.017b	0.287 ± 0.033a
0.05 M	0.167 ± 0.015b	0.186 ± 0.015b	0.222 ± 0.018b
0.01 M	0.083 ± 0.09c	0.051 ± 0.04c	0.022 ± 0.006d

Means followed by the same letter in the same column are not significantly different p=0.05, n=30

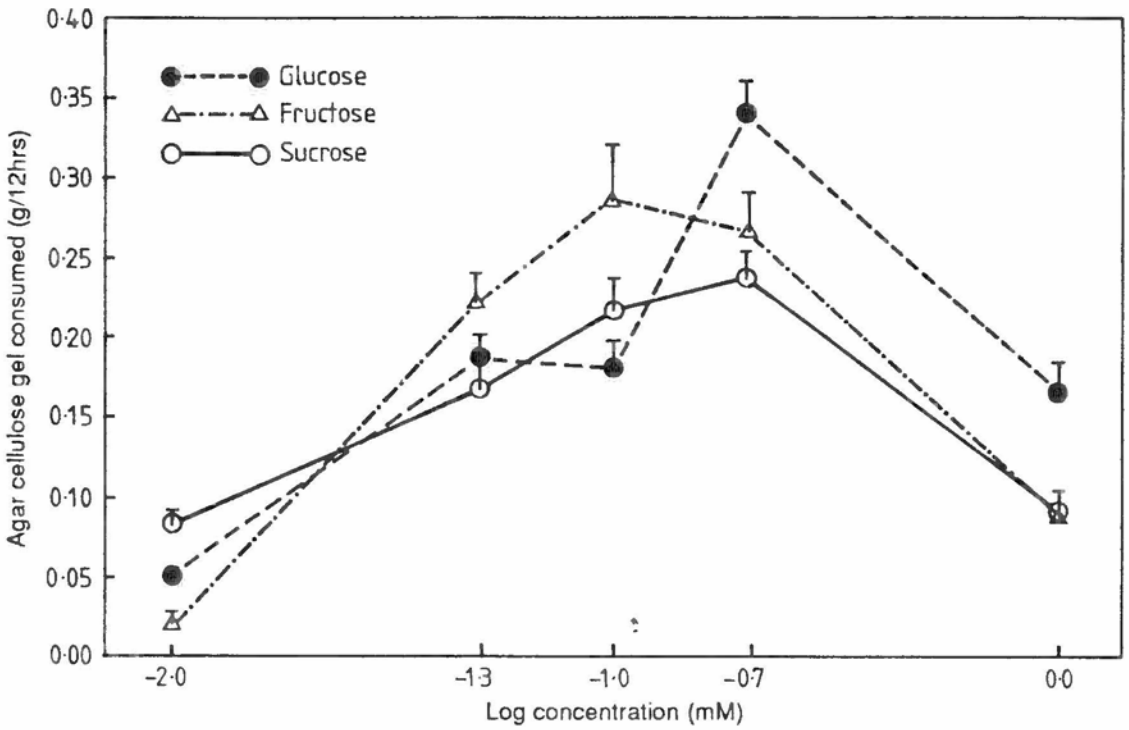


Figure 3: Feeding responses of the 4th instar *M. testulalis* to sucrose, glucose and fructose incorporated in agar cellulose gel.

6.4 Discussion

Bioassays for gustatory stimuli are based on biting and feeding responses of the test animals to a substrate that is incorporated with chemical stimulants and the quantity of ensuing feeding correlates with the level of the incumbent stimulus (Hsiao, 1974). Seed powder elicited the best feeding response among host plant powders suggesting that it contained the most phagostimulants. This observation was not in conformity to earlier results which showed maximum feeding response on flowers and pods. Moreover, the larvae were able to feed on powders from cotton leaves, stems, and bolls and seeds which they had rejected in a fresh form while there was no feeding completely on the flowers. These results are similar to observations made by Hsiao (1974) who noticed that the bettle *Leptinotarsa rubiginosa* did not feed on *Solanum rostratum* and *Solanum heterodoxum* as fresh leaves but readily accepted them in a medium containing leaf powders of these plants. Hsiao and Fraenkel (1968b) have suggested that when leaf powders, but not fresh plants, are acceptable, the plant may contain volatile repellents that are lost in producing the powder. The fact that larvae were able to feed on the cotton leaves and flowers which they had rejected in a fresh form indicates that either the deterrent materials in the plant are volatile and had

been eliminated by drying or the amounts of the powder that were incorporated in the agar were not sufficient to produce a high deterrent effect. On the other hand, the reduced feeding response evoked by host plant pod and flower powders was probably due to the chemical degradation and loss of phagostimulative materials through the drying process but the seeds and leaves appeared to retain their phagostimulants. The method used to prepare the plant material powders is therefore not effective as a bioassay for evaluating the chemical factors influencing feeding responses of an insect.

Dose response curves for sucrose, glucose, and fructose as shown in this study indicate that the amount of agar ingested increased as the concentration increased to 0.2 M above which the amounts ingested decreased. This is a typical response pattern to phagostimulants and has been reported elsewhere (Barton Browne, 1975; Cook, 1976; Dethier, 1976; Ladd, 1986, 1988). Such sugars, sucrose, fructose and glucose are most likely part of the components in the host plant chemical mixture that act as phagostimulants for *M. testulalis*. But as hinted by Ma (1972) and Bernays and Simpson (1982), caution is required in the interpretation of such results with pure compounds because when they are combined with other compounds the insect response may be different.

CHAPTER 7

FEEDING AND ELECTROPHYSIOLOGICAL BIOASSAYS USING FIFTH INSTAR *M. TESTULALIS* LARVAE

7.1 Feeding bioassays of pure compounds, host and non-host plant extracts

7.1.1 Introduction

Feeding bioassays were aimed at finding out how the fifth instar of *M. testulalis* responded to certain known compounds and aqueous extracts from host and non-host plants when presented on an inert substrate. The electrophysiological bioassays were aimed at finding out how the maxillary palp and styloconica sensilla of the same instar responded mainly to some known compounds and water soluble components of the plant extracts.

Previous work on feeding behaviour of *M. testulalis* does not give adequate information on sensory stimuli from host and non-host plant components and there is very little electrophysiological data on the taste sensilla responses of this pest. This study was thus undertaken to investigate the following:

1. Examine the external morphology of the palpal and maxillary galeal and antennal sensilla using scanning electron microscopy.
2. Assess the sensitivity of the taste receptor cells to materials tested.
3. Attempt to relate the results of this bioassay to the feeding bioassay results.

7.2 Materials and methods

7.2.1 Experimental animals

Fifth instar larvae were used in these studies. They were selected as fourth instars and placed in plastic sandwich boxes 17 x 12 x 6 cm (see Plate 8) containing fresh tender cowpea pods which served as food source. After feeding for 2 days the larvae moulted to fifth instar stage. The food and containers were changed daily.

7.2.2 Scanning electron microscopy

Fifth instar larvae were dropped in warm water at ca. 60°C for about 1 minute. This gives them a shock and instant death. The resultant shock extrudes the sensilla outwards (Waladde, Personal communication). Larvae were then kept in 70 % alcohol and sonicated for 3 minutes to remove debris from the mouth parts.

To facilitate thorough cleaning of the specimen, the maxillae bearing the galea and palp were carefully dissected out, tied in a muslin cloth and suspended in a soxhlet apparatus where the wax coating on the sensilla was extracted with hot ethyl acetate for at least 2-3 hours (Waladde, 1977). The specimens were then picked up with a camel hair brush, mounted on stubs and coated with carbon and gold palladium and observed in a Joel scanning electron microscope.

7.2.3 Feeding bioassay

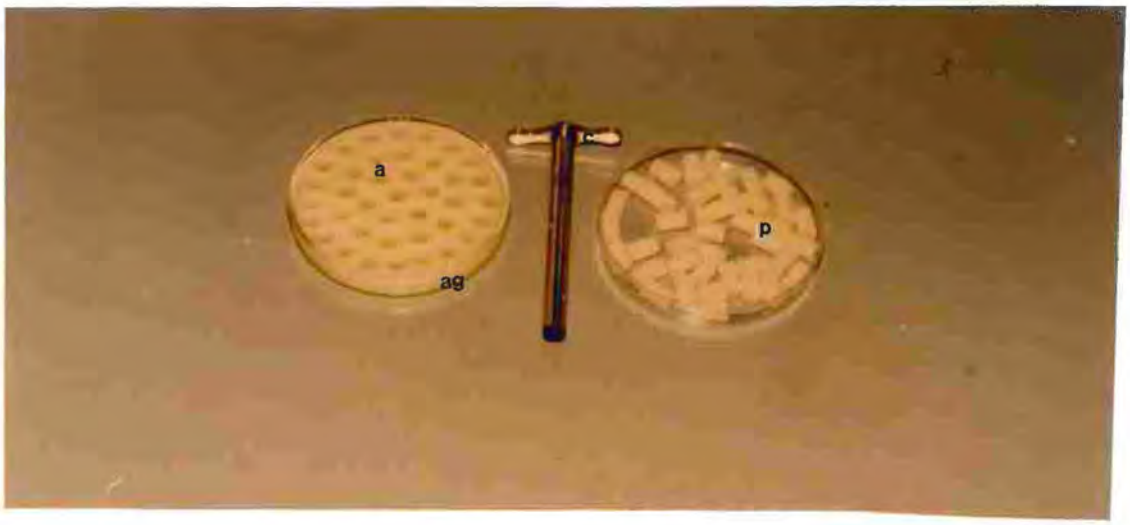
Feeding responses to known compounds (sucrose, methionine, glutamine, glycine, nicotine and tomatine) and to host and non-host plant extracts were tested using 18mm diameter white cellulose acetate membrane discs (Cat. No. C045A047A, pore size 0.45um, obtained from Micro filtration systems, Japan). They were cut with a cork borer No.12. Each disc was dipped once in the solution to be tested. It was then inserted in small vial measuring 39 mm long and 8 mm (internal diameter). Control discs were dipped in distilled water. The vials were specifically suited for this purpose because they resembled the shape of the host plant pods. Early fifth instar larvae starved for 24 hours were used in these studies. One larva was placed in each vial and capped with a lid bearing a fine

netting material which allowed adequate ventilation. The loaded vials were placed in a rack and placed in a sandwich box whose floor was lined with a wet paper towel to increase humidity in the chamber. The sandwich box was then covered with a lid bearing a net Larvae were allowed to feed for 24 hours at laboratory temperature (22-28° C) and humidity (50-70 %). At the end of this period the area of the disc eaten was estimated using a 18 mm diameter piece of graph paper bearing 1 mm^2 grids. Fifteen larvae were used in each experiment. Means of the area eaten were plotted to obtain dose response relationships.

7.2.4 Electrophysiological bioassays

7.2.4.1 Preparation of stimulating solutions

Known compounds tested in the electrophysiological bioassays were glutamine, methionine and sucrose. Each was dissolved in 100mM NaCl. The amino acids were made into concentrations which ranged from 3.25 - 100 mM. Sucrose concentrations ranged from 5.2 - 0.325 %. Extracts were made from lyophilized material prepared as described in section 3.4.4. They were dissolved in 50mM NaCl at concentrations ranging from



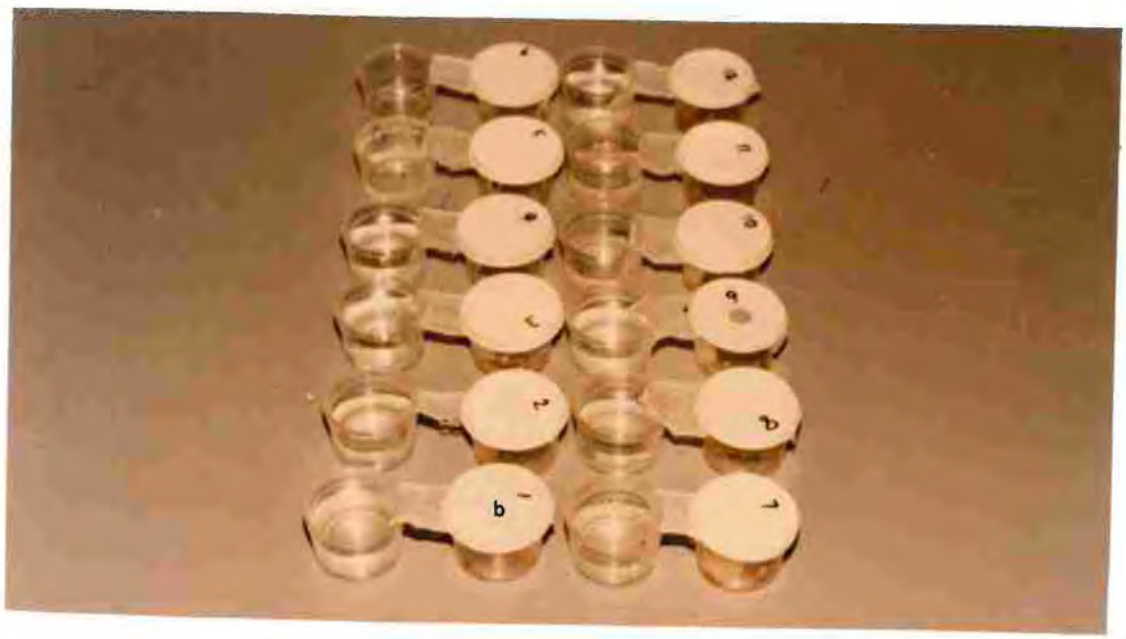


Plate 9a: Agar gel medium in a glass Petri dish (ag) and the cork borer (c) for making agar gel pellets (p).

b: Plastic cups with snap-on lids for testing feeding responses of fourth instar larva to various stimuli incorporated in agar gel pellets.

conical flask and autoclaved in a 280 EH Rustrak® autoclave at 115° for 1 hour, then cooled to about 40° C. To make the test medium, 4g plant powder was added to the agar cellulose medium and stirred thoroughly before pouring it in a glass Petri dish (6 cm diameter) to set. Sugars tested for feeding response included sucrose, glucose and fructose. The agar medium for testing sugars was prepared as detailed above but sugar was added to it instead of the plant powder. The sugars were weighed according to their molecular weights to make 0.01, 0.05, 0.1, 0.2, and 1 molar concentrations which were poured in glass petri dishes to set. A cork borer (No.6) was used to punch out cylindrical pellets (Plate 9a) whose volume was obtained by the Archimedes principle using a graduated centrifuge tube. The experimental chamber consisted of small plastic cups with snap-on lids. Sides of the cup were perforated with small holes for ventilation. In each cup, 1 pellet and two fourth instar larvae previously starved for 24 hours were placed and were allowed to feed for 12 hours. Control insects were offered plain agar. A small strip of muslin cloth was laid on top of the cup leaving about 6 cm trailing outside. The trailing part was dipped in another cup containing water to form a wet bridge over the capsule before placing lid on top (Plate 9b). This arrangement maintained a steady supply of moisture

Cotton leaves and flowers supported larval growth up to second instar. Development was, nevertheless, possible on cotton bolls but mortality was very high and only 20 % of the larvae pupated. Furthermore, pupal weights were lower than those of larvae reared on other host plant parts except on common bean stem.

5.3.4 Role of larval food on the feeding preferences of the fifth instar *M. testulalis* larvae

Larvae reared on cowpea ate significantly less quantities of the common bean pod than cowpea pods. On the other hand, larvae reared on common beans ate significantly more cowpea than common beans when offered in a no choice situation (Table 24, Appendix 5).

Table 23

Growth and developmental rate of larval *M. testulalis* on its host and non-host plant parts.

Plant	Part	S	Mean larval period (days ± S.E)	Mean pupal weight (g.± S.E)	Percent pupa (%)
Cowpea	Leaf	4	17.5 ± 0.27a	0.025 ± 0.03a	13.3
	Stem	*			
	Flower	14	12.5 ± 0.20b	0.046 ± 0.01b	46.7
	Pod	20	12.6 ± 0.18b	0.042 ± 0.02b	66.7
	Seed	14	12.5 ± 0.20	0.042 ± 0.02b	46.7
Common bean					
	Leaves	*			
	Stem	3	19.3 ± 0.33a	0.023 ± 0.03c	10
	Flower	9	12.0 ± 0.28d	0.032 ± 0.02c	30
	Pod	14	13.4 ± 0.13b	0.041 ± 0.01	46.7
	Seed	11	12.5 ± 0.15c	0.036 ± 0.02	36.7
Cotton	Leaves	*			
	Flower	*			
	Boll	6	14.3 ± 0.21	0.024 ± 0.01	20

S: Is the final number of pupa obtained from 30 larvae tested in 3 replicates on the specified plant part.

* All larvae died before pupation

Means followed by the same letter for each plant part are not significantly different (p=0.05, D.M.R.T).

Table 24

Role of larval rearing food on feeding preferences of the fifth instar *M. testulalis*

Rearing food	Tested food	Dry wt pod eaten g ± S.E	C.V*
Cowpea pods	Cowpea pods	0.056 ± 0.03b	30.7
Cowpea pods	Common bean pods	0.036 ± 0.03c	45.9
Common bean pods	Common bean pods	0.077 ± 0.04b	34.0
Common bean pods	Cowpea pods	0.077 ± 0.07a	19.8

C.V*= Coefficient of variation

5.4 Discussion

The order of preference of different parts of cowpea plant was in the following diminishing order: pods > flower > stem > leaves > developing seeds while for common beans it was: pods > flowers > stem > leaves > developing seeds. Clearly, developing pods and flowers of both host plants elicited maximum feeding response as opposed to other parts of the plant while the fourth instar larvae did not feed on cotton leaves and flowers.

According to Beck (1965) the quantity of plant material ingested is a reflection of the stimulus of the diet. Results obtained in this study show that host plant pods and flowers are the most palatable to the fourth instar larvae and therefore are most likely to be containing more phagostimulants as opposed to other parts. Lack of feeding on cotton leaves and flowers suggests that either they contain deterrents or lack phagostimulants.

Common bean leaves elicited very little feeding, probably due to the presence of trichomes which trapped and impeded movement of larvae and thus reduced feeding. Similar effects by common bean leaves

have been reported on *Emposca fabae* (Singh *et al.* 1971), *Melanoplus* species (Smith and Grodowitzi, 1983), *Aphis craccivora* and *Acyrosiphon pisum* (Lampe, 1982) thus suggesting that trichomes are the first line of defence against insect attacks. This could be playing an important role as a source of resistance observed in this variety of common bean against *Maruca* infestation.

Significantly higher feeding observed on pods of both host plants could be a reflection of high levels of phagostimulants incumbent in the tissues. This trend was also reflected in the number of insects that fed on these parts. Cowpea seeds, stem and leaf and common bean pods and flowers attracted and arrested a higher percentage larvae for feeding in the choice test (Table 21). This was an indication that these parts contained more phagostimulants. From Table 20, it is apparent that the common bean is a "delicious" host for *M. testulalis* but it is protected by the leaves which were found to deter larval feeding. Thus if the larva can only overcome the defensive effect of the leaves the common bean can be even more susceptible than cowpea.

Results on preference index as shown in Table 22 show that the percent larvae that fed on pods and

flowers in both host plants were higher than in the others, suggesting that they offered more feeding stimuli. When the suitability of different parts of the hosts and non-host plant were compared, cowpea was the most suitable host plant for larval growth and development. Flowers, pods and seeds were the most suited parts in this respect. Leaves were the least suitable and larvae took long to grow on it. Moreover, pupal weights were similarly lower. Larval growth and development on common bean parts was generally slower than on cowpea. The fastest growth was observed on flowers and the slowest growth on stem. The leading pupal weight on the other hand, was from larvae reared on pods and seeds while the lowest was on the stem. Growth and development on cotton was only possible on bolls. Nonetheless, larval period was long and mean pupal weight low implying that antibiosis, as suggested by Painter (1951) was probably involved in non-preference of cotton to *M. testulalis* larval feeding. These results show that host plant pods, flowers and seeds contain high levels of nutrients in addition to phagostimulants.

Larvae reared on cowpea ate significantly less on common bean pod than cowpea pods indicating that they preferred cowpea to common bean pod. The fact that larvae reared on common bean ate significantly more cowpea

than common bean when in a no choice situation indicates that the preference for feeding on cowpea had not been lost by rearing the larvae on common bean. This also implied that the preference to cowpea was genetically determined. Previous studies have correlated the presence or absence of induction of feeding preference on a particular plant pair with the taxonomic similarity of those plant species (Jermy *et al*. 1968, deBoer and Hanson, 1984). In *Manduca sexta* it was shown that no induction could be demonstrated when one species of *Solanum* was paired with another, suggesting that an induction is less manifest when larvae are tested in a choice with a close relative of the inducing species (de Boer and Hanson, 1984). These results are very similar to my own findings in spite of the fact that cowpea and common bean belong to different genera. Probably in the two plant species, a similar chemosensory profile is elicited by the phytochemicals of the plants tested. Thus, distantly related species are more likely to elicit a stronger induction of feeding preference than closely related species.

CHAPTER 6

FEEDING RESPONSES OF THE FOURTH INSTAR *M. TESTULALIS* LARVAE TO AGAR PELLETS CONTAINING HOST AND NON-HOST PLANT POWDERS AND TO KNOWN PHAGOSTIMULANTS

6.1 Introduction

The feeding responses of phytophagous insects is influenced by many factors of which the physical and chemical factors are most important (Saxena, 1969; Norris and Kogan, 1980). In the previous chapter, it was shown that host plant pods and flowers evoked more larval feeding than other parts while non-host plant parts elicited no feeding. As a follow up to these studies, the role of chemical factors from the plant and that of sugars: sucrose, glucose and fructose, which are known to have a phagostimulative effect in many insects, were examined.

6.2 Materials and methods

Leaves, stems, flowers, pods and seeds of cowpea and common bean and cotton leaves and flowers were harvested and dried in open air in the laboratory for 3 weeks. A Glen Creston™ electric grinder was used to grind each part separately. An agar medium was made by dissolving 4g agar in 100 ml distilled water in a

Table 21

Feeding preference of first instar *M. testulalis* larvae on different parts of cowpea and common bean.

Plant part	Host plant		Both parts	Neither part
	Cowpea	Common bean		
Leaves	53	41	6	0
Stems	45	21	23	11
Flowers	40	54	6	0
Pods	43	57	0	0
Seeds	60	40	0	0

Table 22

Feeding preference of first instar *M. testulalis* larvae on different parts of cowpea.

Test part offered		% Insects feeding				
A	B	A	B	A+B	Neither A or B	P.i.B*
Leaf	Stem	31.0	55.2	13.8	0	24.2
Leaf	Flower	27.6	65.5	6.89	0	37.9
Leaf	Pod	17.9	64.3	10.7	7.1	46.4
Leaf	Seed	39.3	50.0	10.7	0	10.7

P.i.B* = Preference index for B: Calculated as a percentage difference B-A. It represents a feeding preference for B over A

5.3.3 Suitability of host and non-host plant parts for larval growth and development

Larval development on cowpea leaves was significantly longer than on flowers, pods and seeds. Duration of the larval period on cowpea flowers, pods and seeds was the same (Table 23, Appendix 1). All larvae reared on cowpea stem died before reaching their 3rd instar stage. The highest percent pupa (66.7 %) was recovered from larvae reared on pods and 46.7 % pupa was obtained from larvae reared on seeds and flowers. Mortality was highest in the larvae reared on leaves and only 13 % pupa were recovered. Mean pupal weights of the larva reared on pod, flowers and seed were similar but that of the leaf was significantly smaller (Appendix 2).

On the common bean, flowers and seeds were the most suitable for larval development (Table 23, Appendix 3). This was followed by pods, and stem was the least suitable. Larvae reared on leaves were killed by the plant trichomes before they moulted into second instars. On the stems, only 10 % of the larvae pupated while the highest percent pupa were recovered from larvae reared on pods (46.7%) and on seeds (36.7 %). Pupae from larvae reared on pods and seeds had similar mean weights but those from larvae reared on stem had significantly low weights (Appendix 4).

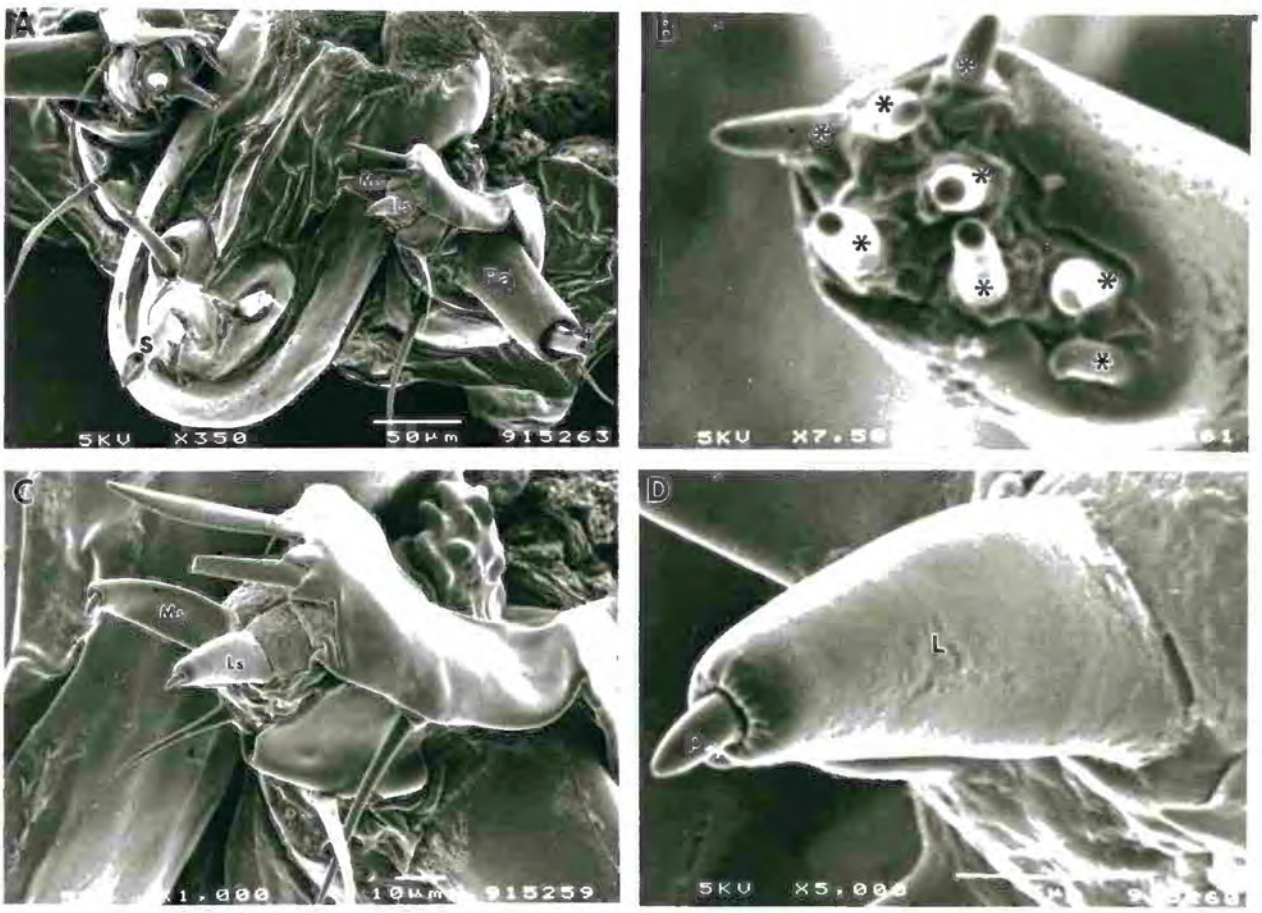
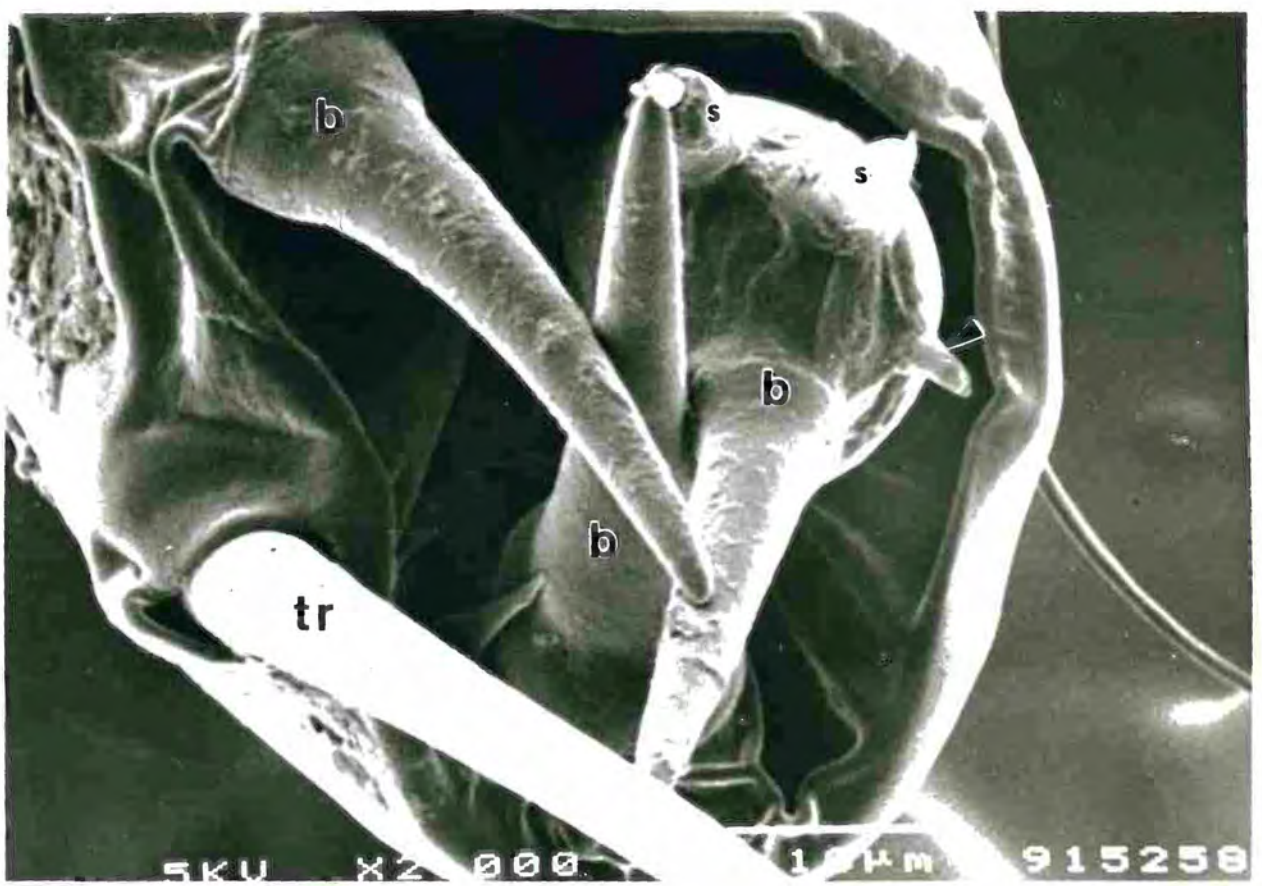

A
S
5KV X350 50µm 915263
B
5KV X7.5
C
Ms
Ls
1,000 10µm 915259
D
L
5KV X5,000

Plate 11: Scanning electron micrograph showing three basiconica (b), one trichodea (tr) and at least 2 styloconica (s, arrowhead) sensilla on the terminal antennal segment of the fifth instar *M. testulalis* larva.


b
s
s
b
b
tr
5KV X2 000
915258

7.3.2.2 Amino acids

Feeding responses to amino acids were in the following order of preference: Glutamine > Methionine > Glycine (Fig. 6). Methionine (Appendix 11) and glutamine (Appendix 12) stimulated feeding at very narrow concentration range of 6.25 - 12.5 mM. Concentrations above this had negative effects on feeding. Hardly any feeding was evoked by glycine Appendix 13).

7.3.2.3 Alkaloids

Among the alkaloids, tomatine and nicotine elicited feeding responses below that of the controls (Fig. 7, appendices 14, 15). Nicotine in addition caused very high mortality in all doses tested but tomatine did not kill the larvae.

7.3.2.4 Plant extracts

n-hexane and methanolic extracts of the cowpea leaf evoked relatively similar feeding responses and the larvae did not respond decisively to increasing concentration of these extracts (Fig. 8, Appendices 16, 17). On the other hand, most of the aqueous extracts from the different parts of the host plants evoked positive feeding responses. Aqueous extracts from cowpea and common bean pods and flowers (Fig. 9A,

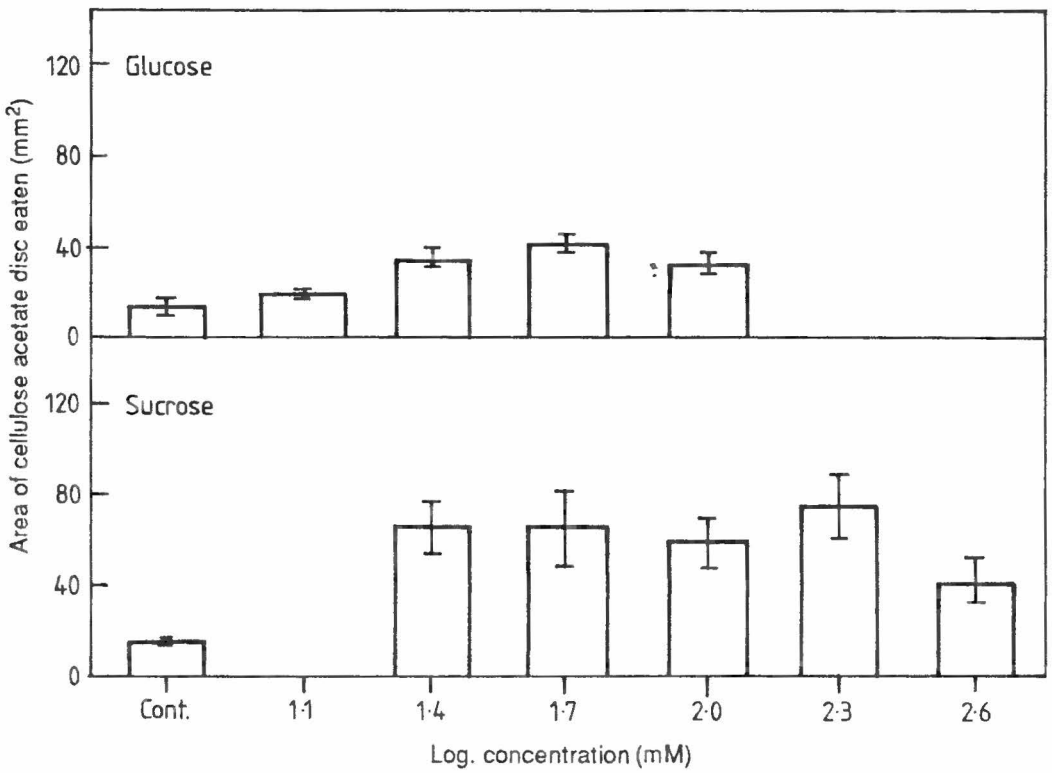


Figure 5: Feeding responses of the fifth instar *M. testulalis* larvae to glucose and sucrose impregnated in cellulose acetate paper discs.

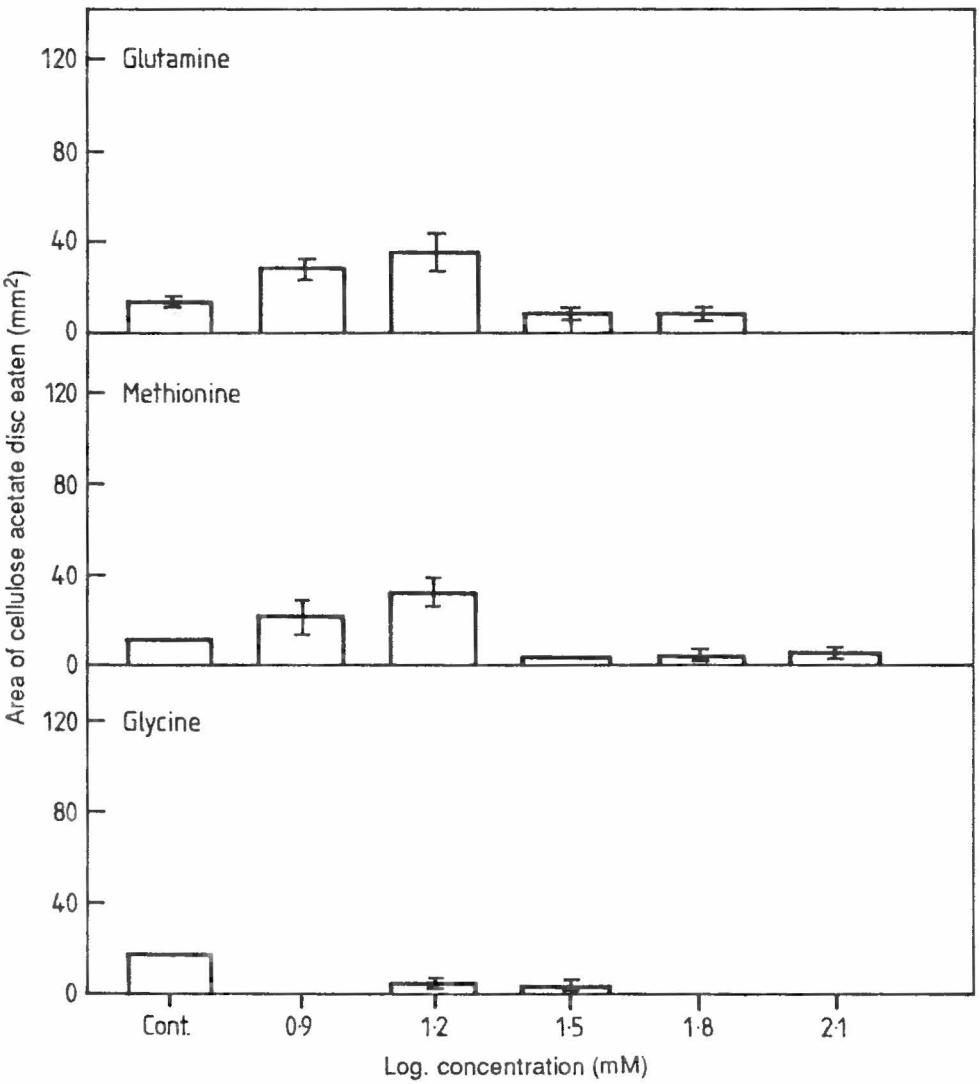


Figure 6: Feeding responses of the fifth instar *M. testulalis* larvae to glutamine, methionine and glycine impregnated in cellulose acetate paper discs.

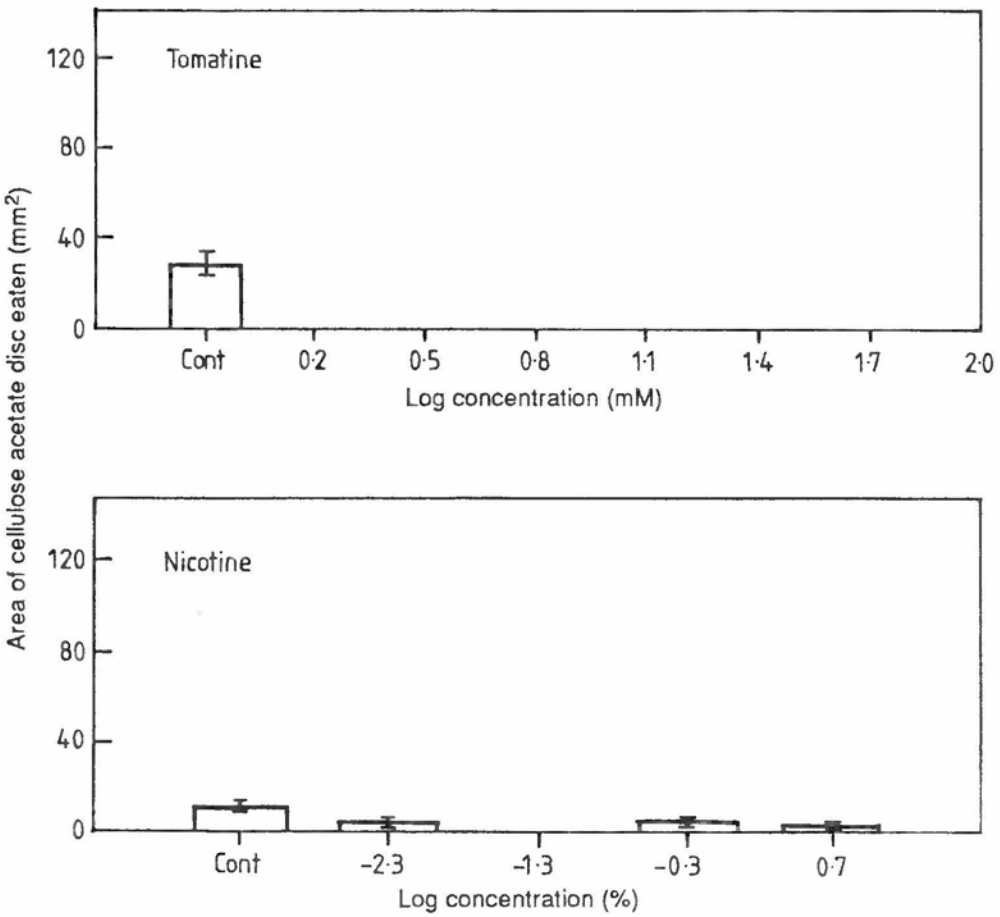


Figure 7: Feeding responses of the fifth instar *M. testulalis* larvae to tomatine and nicotine impregnated in cellulose acetate paper discs.

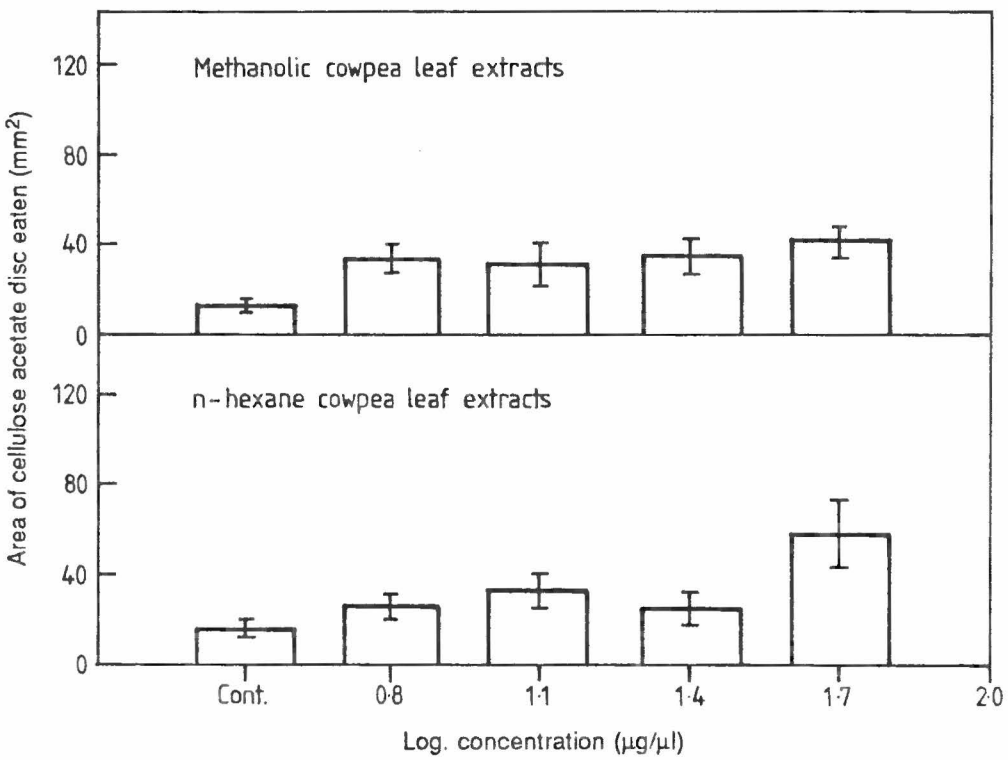


Figure 8: Feeding responses of the fifth instar *M. testulalis* larvae to n-hexane and methanolic cowpea leaf extracts impregnated in cellulose acetate paper discs.

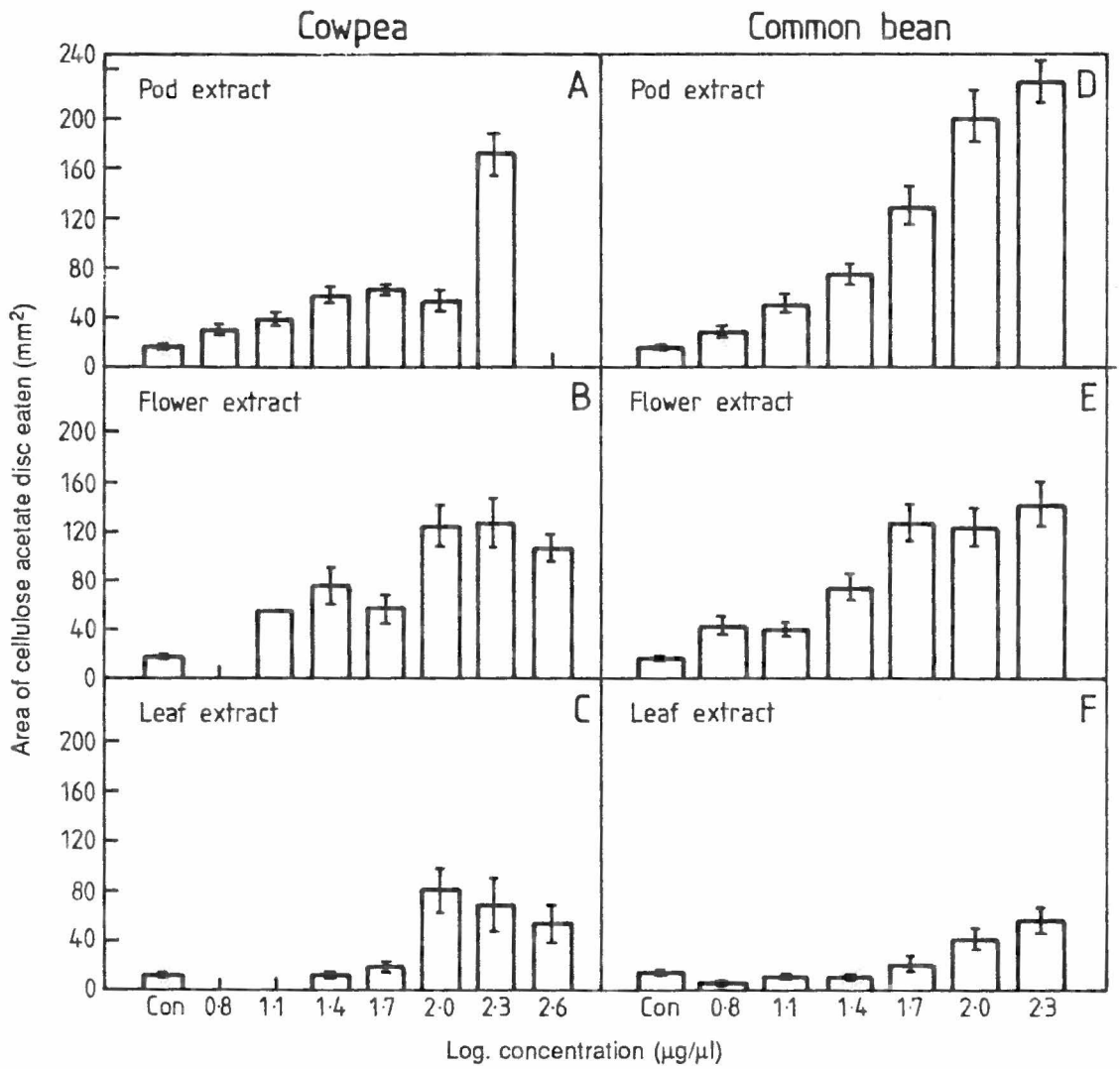


Figure 9: Feeding responses of the fifth instar *M. testulalis* larvae to aqueous extracts of cowpea (A-C) and common beans (D-F) impregnated in cellulose acetate paper discs.

B, D and E , Appendices 18, 19, 20, 21) elicited better feeding than leaves of both host plants (Figs. 9C and 9F, Appendices 22, 23). Feeding responses to cotton leaf extracts (Fig. 10, Appendix 24) were very poor but cotton flower extracts evoked responses similar to those elicited by the host plant flower extracts (Appendix 25).

7.3.3 Electrophysiological responses of the palp and styloconica sensilla to various test stimuli

7.3.3.1 Sodium chloride

Palpal sensilla were insensitive to fairly low concentrations of sodium chloride. Concentrations between 1 - 25 mM evoked variable firing patterns. However, when the concentration rose from 50 -100 mM reproducible changes in the spike frequency were observed (Fig. 11A, Appendix 26). Therefore, 100 mM concentration was used as the electrolyte in which known compounds were dissolved while 50 mM concentration was used to dissolve plant extracts.

Dose response curves of the medial and lateral sensilla styloconica in response to sodium chloride

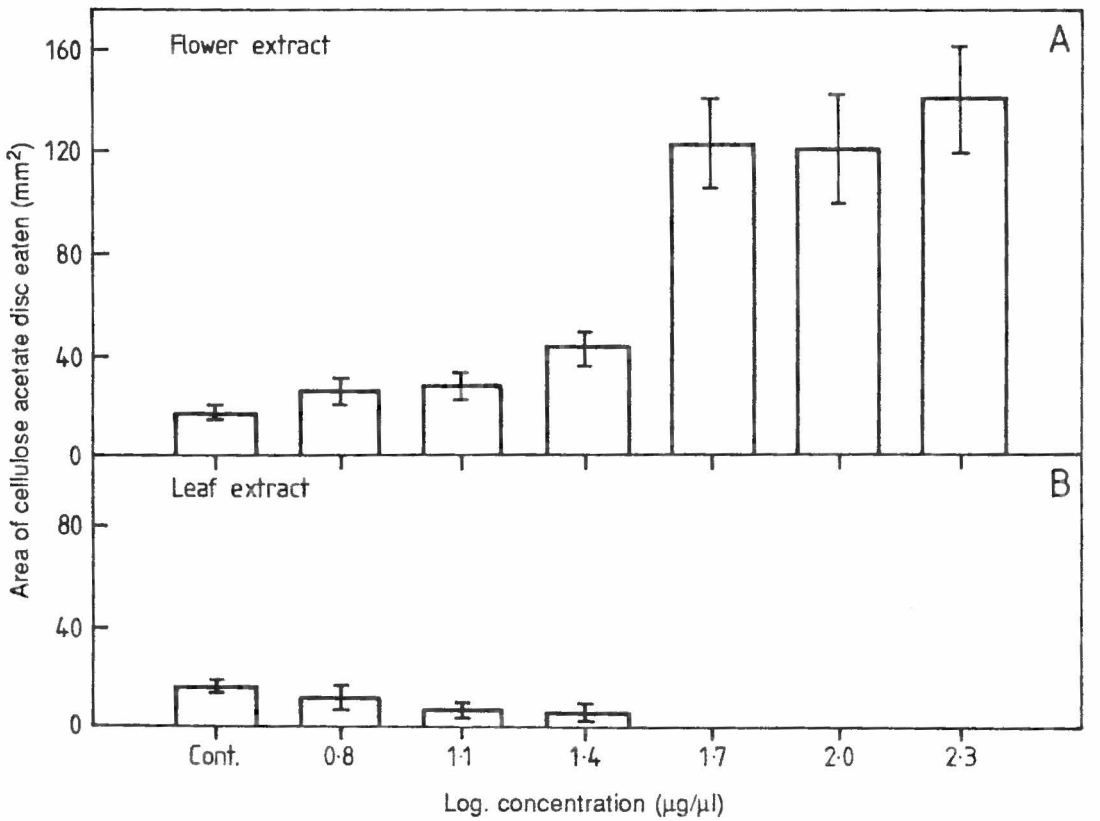


Figure 10: Feeding responses of the fifth instar *M. testulalis* larvae to aqueous extracts from cotton flowers (A) and leaves (B) impregnated in cellulose acetate paper discs.

were similar. (Appendix 27, 28) Spike frequency increased with increase in concentration levelling off at 50 - 100 mM (Fig. 12A). The medial sensilla showed a higher sensitivity to NaCl than the lateral sensilla and only one cell type was stimulated.

7.3.3.2 Sugars

Concentration of sucrose between 0.325 - 1.3 % evoked no clear spike firing from the palpal sensilla but concentrations above 1.3% depressed the cells from firing while 5.2 % sucrose inhibited the sensilla from firing (Fig. 11B, Appendix 29). The response from the sensilla was multineuronal (Plate 12).

The lateral sensilla was more sensitive to sucrose than the medial sensilla (Appendix 30). Moreover, the dose response curves were also different. Sucrose depressed cells in the medial sensilla (Appendix 31) while in the lateral sensilla it triggered an increase in spike frequency with increasing concentration up to 1.3 % (Fig. 12B). The response evoked in the lateral sensilla was multineuronal (Plate 13).

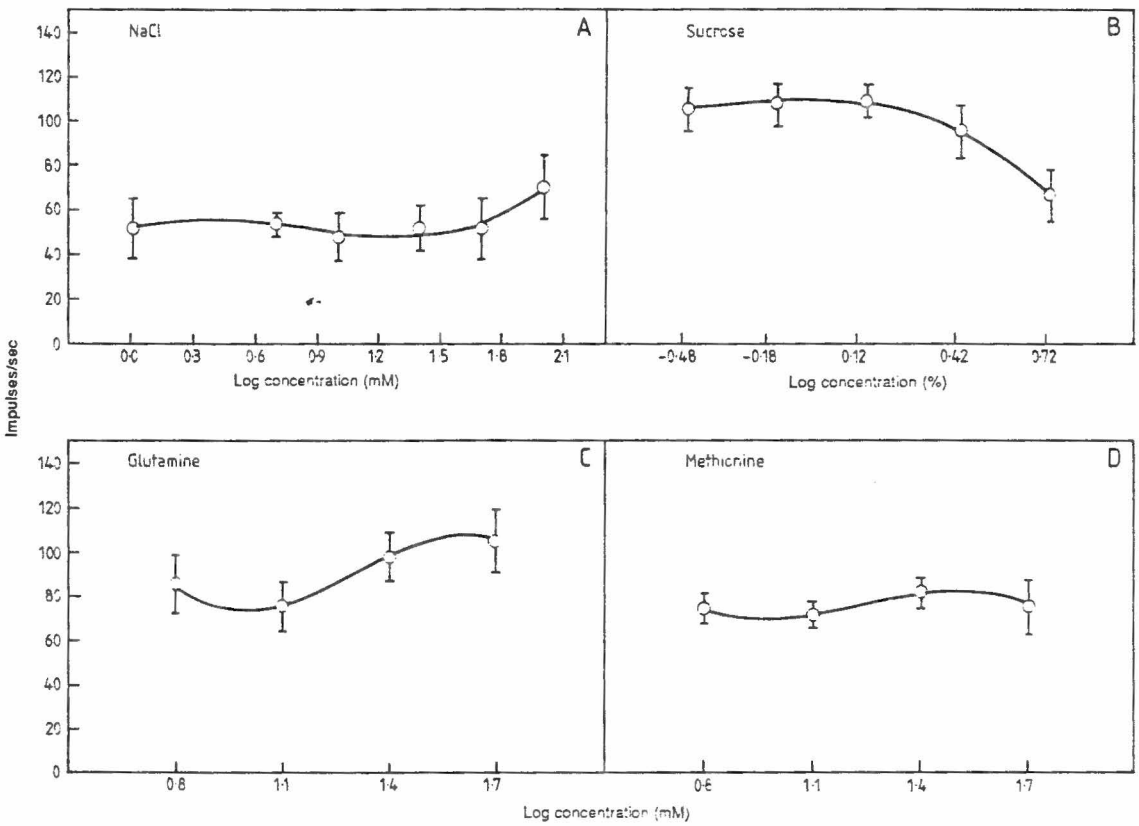


Figure 11: Electrophysiological dose response curves of gustatory cells in the maxillary palp sensilla of the 5th instar *M. testulalis* stimulated with (A) NaCl, (B) sucrose, (C) glutamine and (D) methionine.

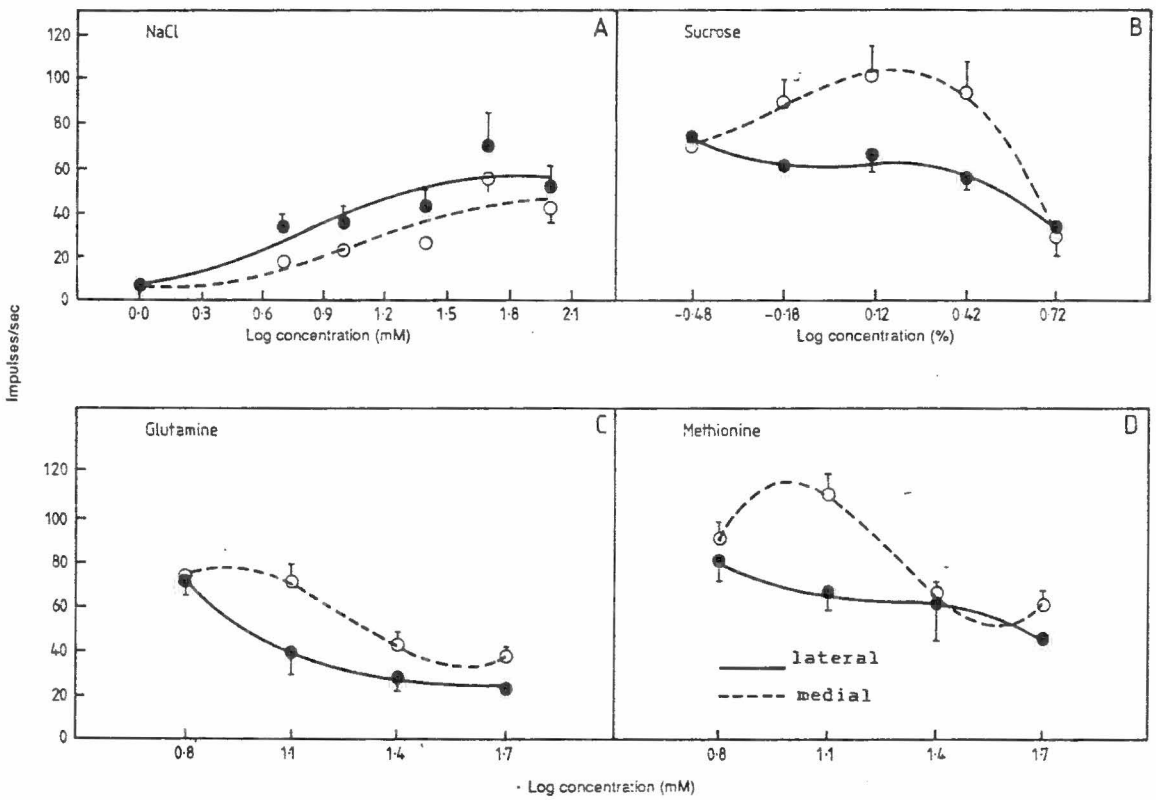


Figure 12: Electrophysiological dose response curves of gustatory cells in lateral and medial styloconica sensilla of the 5th instar *M. testulalis* stimulated with (A) NaCl, (B) sucrose, (C) glutamine and (D) methionine.

Plate 12: Spike patterns recorded from the maxillary palp sensilla of the fifth instar *M. testulalis* stimulated with a series of increasing concentrations of sucrose.

(A) 100mM NaCl

(B) 0.325 %

(C) 1.25 %

(D) 2.6 %

(E) 5.2 %

(F) 100mM NaCl

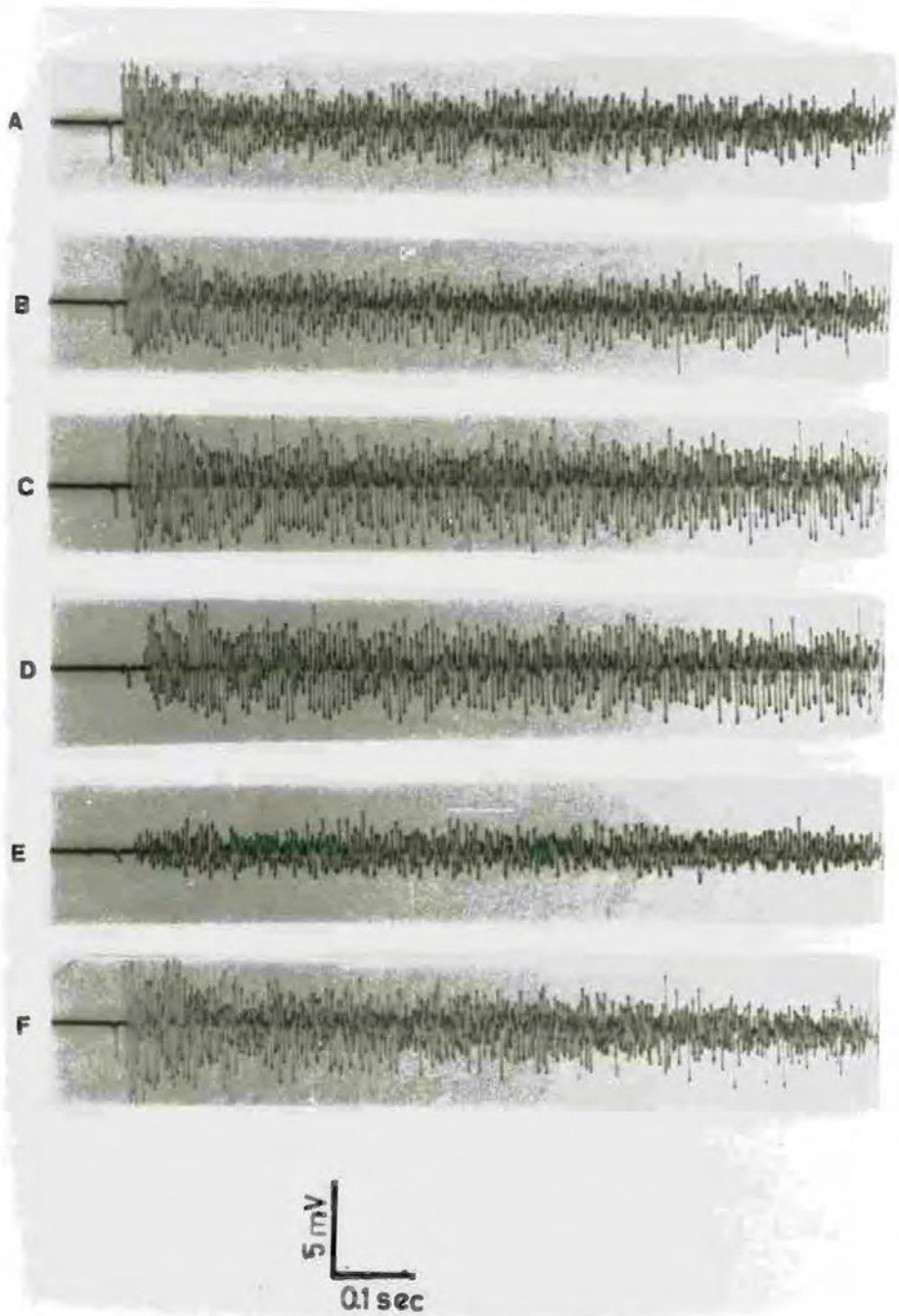

A
B
C
D
E
F
5 mV
0.1 sec

Plate 13: Spike patterns recorded from the lateral styloconicum sensillum of the fifth instar *M. testulalis* stimulated with a series of increasing concentrations of sucrose.

(A) 100mM NaCl

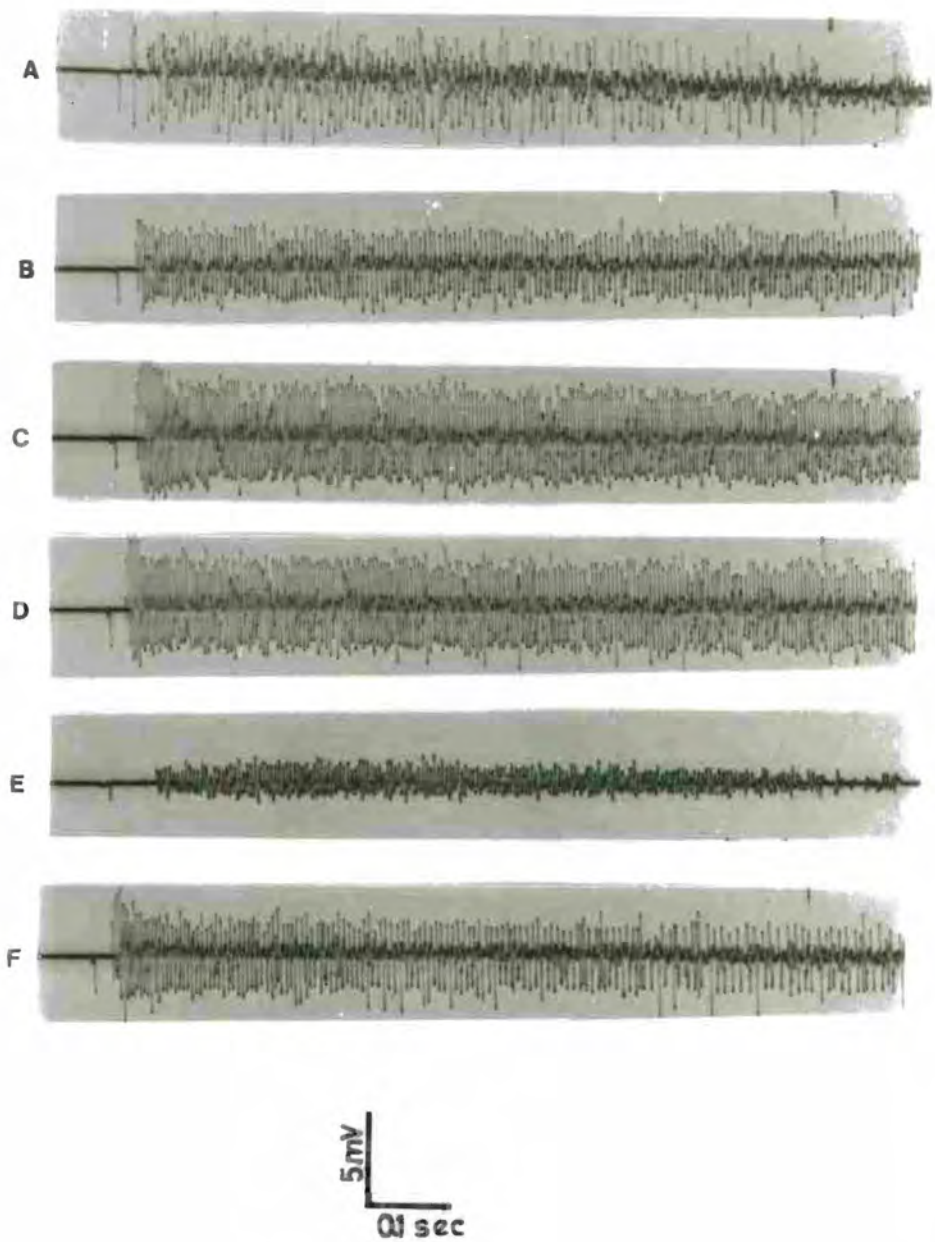
(B) 0.325 %

(C) 1.25 %

(D) 2.6 %

(E) 5.2 %

(F) 100mM NaCl


A
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5mV
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Plate 14: Spike patterns recorded from the medial styloconicum sensillum of the fifth instar *M. testulalis* stimulated with a series of increasing concentrations of methionine.

(A) 100mM NaCl

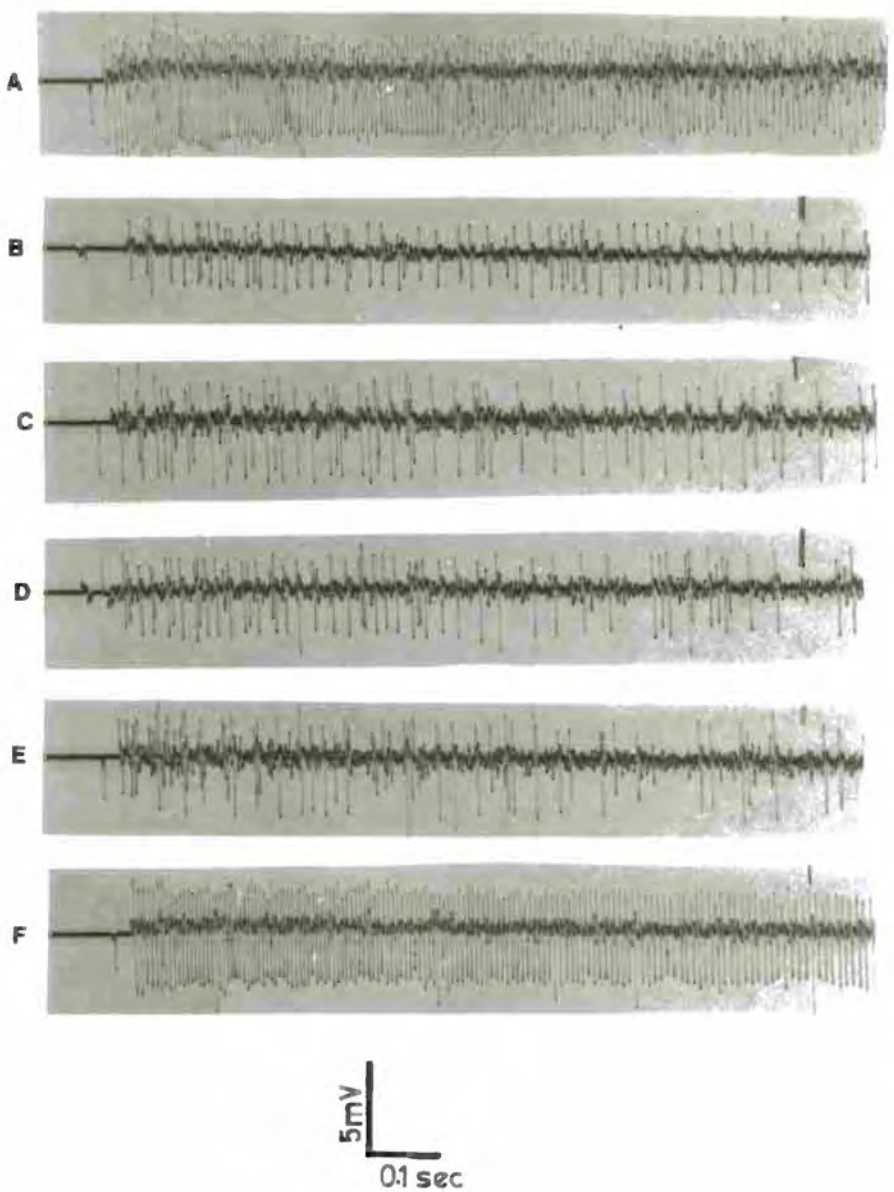
(B) 6.25 mM

(C) 12.5 mM

(D) 25 mM

(E) 50 mM

(F) 100mM NaCl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E
F
5mV
0.1 sec

7.3.3.3 Amino acids

The palpal sensilla were more sensitive to glutamine than to methionine, with a distinct increase in spike frequency with increasing concentration (Fig. 11C, Appendices 32, 33).

Dose response curves evoked by glutamine and methionine on the styloconica sensilla were quite similar. Spike frequencies in response to methionine were however higher than those evoked by glutamine (Fig. 12C and 12D). Glutamine appeared to depress cells of the lateral and medial sensilla (Appendix 34, 35). The lateral sensilla were however more sensitive to both amino acids than the medial sensilla. Methionine evoked an increase in spike frequency within a narrow range between 6.25 - 12.5 M. in the lateral sensilla (Appendix 36) but depressed the firing of cells in the medial sensilla and at least 2 cell types were stimulated (Plate 14, Appendix 37).

7.3.3.4 Plant extracts

Cowpea pod and flower extract evoked slowly adapting responses in the palpal sensilla and at least 4 cell types were active but it appeared that the leaf extract stimulated less than 3 cells. Increasing

concentrations of pod extract depressed the activity of the sensilla (Appendices 38, 39) but leaf extracts (Appendices, 40) evoked responses that were almost half the value of the pod extracts (Fig. 13). There was no clear concentration response to the flower extracts and a five fold concentration was barely distinguishable from the most dilute concentration in both host plants.

The lateral sensilla was more sensitive than the medial sensilla to all cowpea extracts. Pod and flower extracts (Fig. 14A and 14B Appendix 41, 42) elicited higher impulse frequencies in the lateral sensilla compared to the leaf extracts (Fig. 14C, Appendix 43). Furthermore, the leaf extract depressed the firing from cells of the lateral and medial sensilla. The pod and flower extract elicited 3 slowly adapting amplitude spikes in the lateral and medial (Plates 15, 16 and 17). All responses were slowly adapting. All extracts however appeared to depress the activity of the medial sensilla particularly the cowpea leaf extract (Plate 18, Appendices 44, 45, 46).

Dose response curves of the palpal sensilla to aqueous extracts of common bean were similar to those elicited by the cowpea extracts. Pods and flower extracts (Appendices 47, 48) clearly evoked higher

impulse frequency responses than leaf extracts (Fig. 13 A, B, C, D, E and F, Appendix 49). Pod extracts from both host plants evoked spike response patterns whose slope was similar suggesting that their functions were the same.

Activity of cells in the lateral and medial sensilla in response to common bean extracts were also quite similar to those of cowpea extracts particularly with regard to the pod and flower extracts (Fig. 14 A, B, C, D, E, F). The lateral sensillum (Appendices 50, 51) was again more sensitive than the medial sensilla to the extracts (Appendices 52, 53). Three cells with slowly adapting characteristics were generated in the lateral sensilla (Plate 19) and at least 2 in the medial styloconica sensilla (Plates 20 and 21) in response to the pod and flower extracts respectively. The total number of spikes generated in response to the leaf extract (Appendices 54, 55) were clearly lower than those from flower and pod extracts in both lateral and medial sensilla. This is depicted in the slopes of their curves.

Cotton flowers and leaves elicited relatively similar spike frequency responses from the palpal sensilla. Both extracts depressed the activity of the

palpal sensilla (Fig 15A and B, Appendix 56, 57). The response was multineuronal with at least 3 spike heights.

Cotton leaf and flower aqueous extracts also depressed the firing from the cells in the lateral sensilla and medial sensillum (Fig. 16). The lateral sensillum (Plate 22,) was more sensitive to the leaf extract (Appendix 58) than the medial sensillum (Appendix 59, Plate 23). The flower extract severely depressed the activity of the medial sensillum (Plate 24), (Appendix 60) and lateral sensillum (Appendix 61).

7.4 Discussion

In the absence of a phagostimulant, *M. testulalis* larvae hardly fed on cellulose acetate paper. Cellulose acetate paper is thus a very useful bioassay material in this regard. Sucrose evoked a better feeding response than glucose indicating that it was a better phagostimulant. These results thus reaffirm similar observations made by Okech (1986) who conducted his experiments with an agar based bioassay using fourth instar larvae. Electrophysiological recordings carried out to relate these bioassay

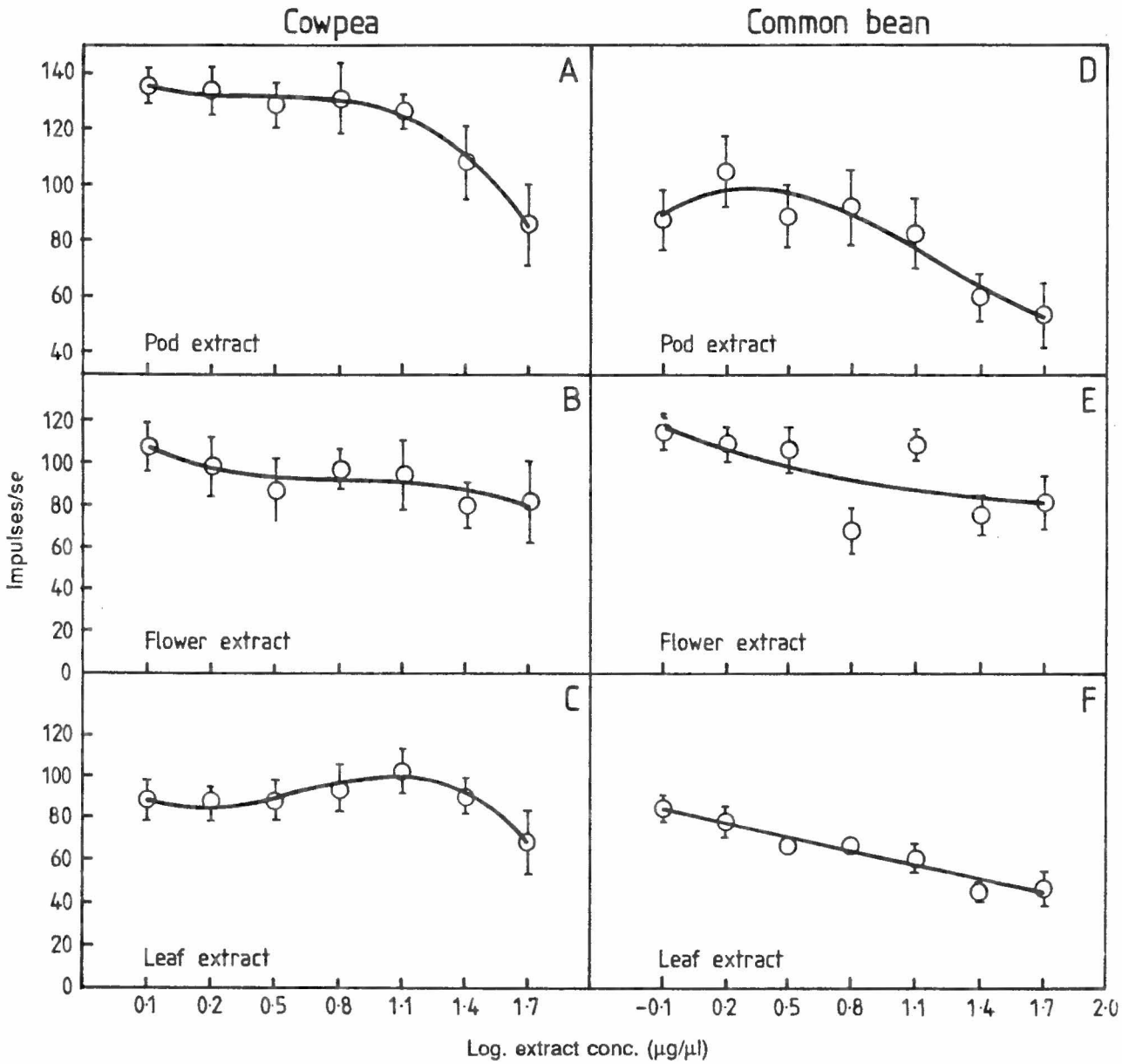


Figure 13: Electrophysiological dose response curves of gustatory cells in the maxillary palp sensilla of the 5th instar *M. testulalis* stimulated with aqueous extracts from cowpea (A-C) and common beans (D-F).

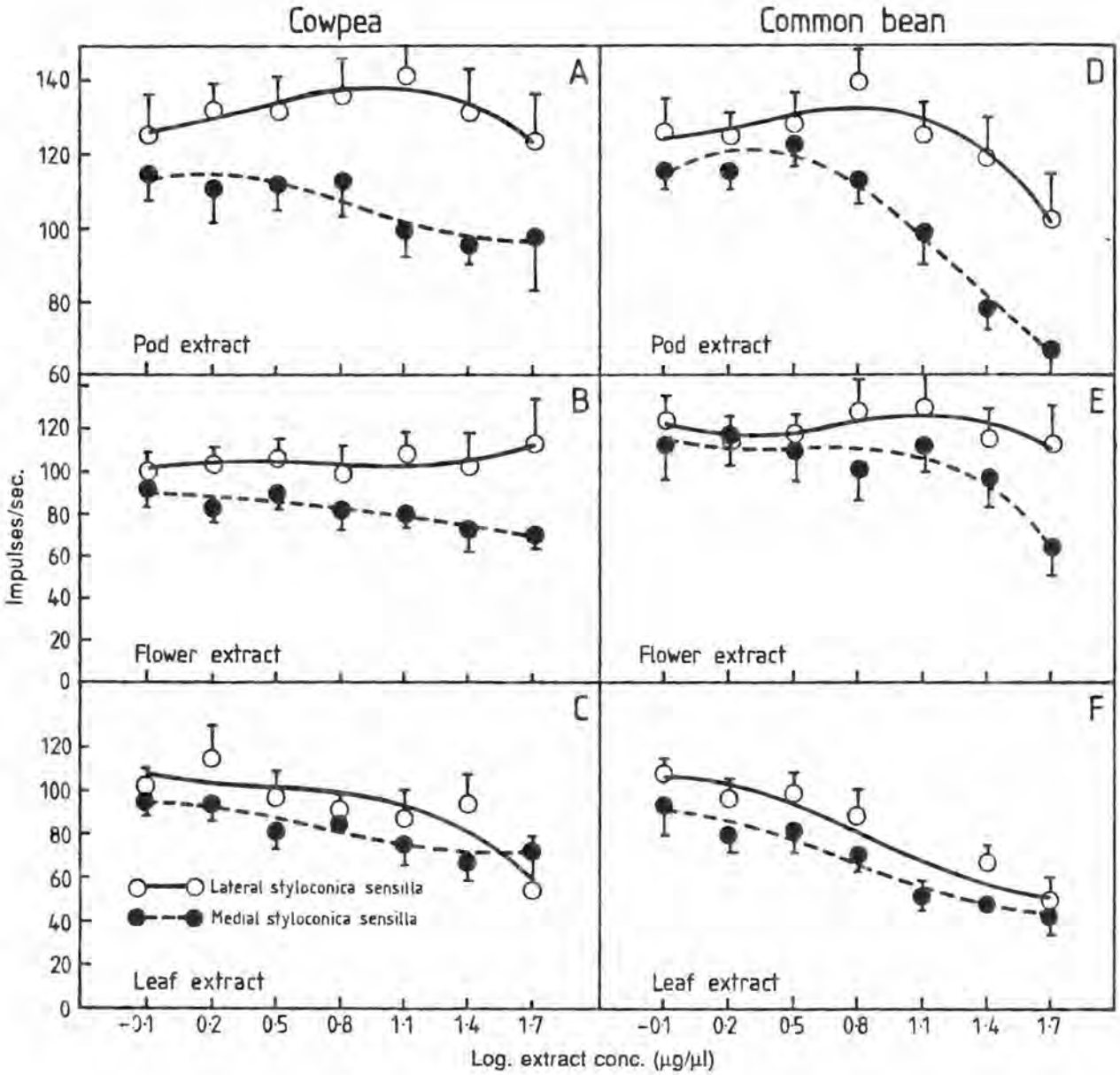


Figure 14: Electrophysiological dose response curves of gustatory cells in lateral and medial styloconica sensilla of the 5th instar *M. testulalis* stimulated with aqueous extracts from cowpea (A-C) and common beans (D-F).

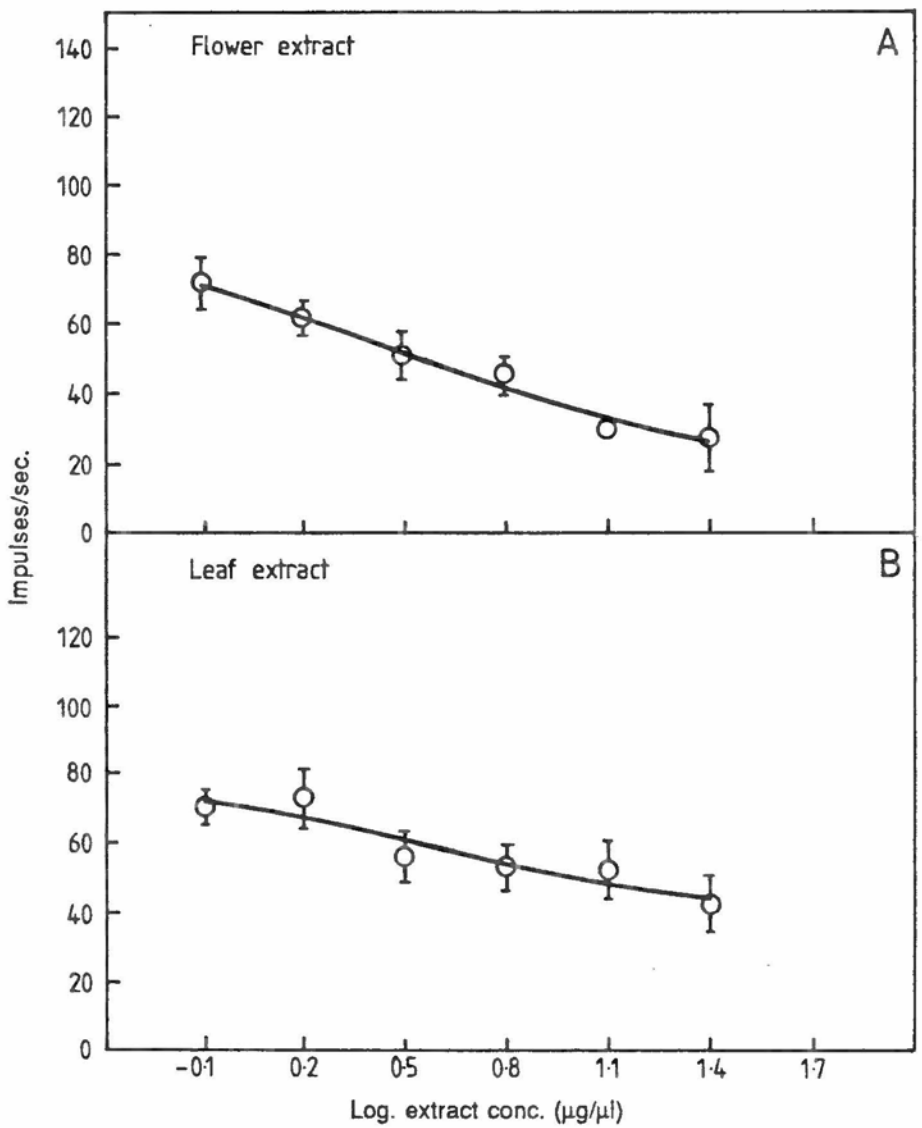


Figure 15: Electrophysiological dose response curves of gustatory cells in the maxillary palp sensilla of the 5th instar *M. testulalis* stimulated with aqueous extracts from cotton flowers (A) and leaves (B).

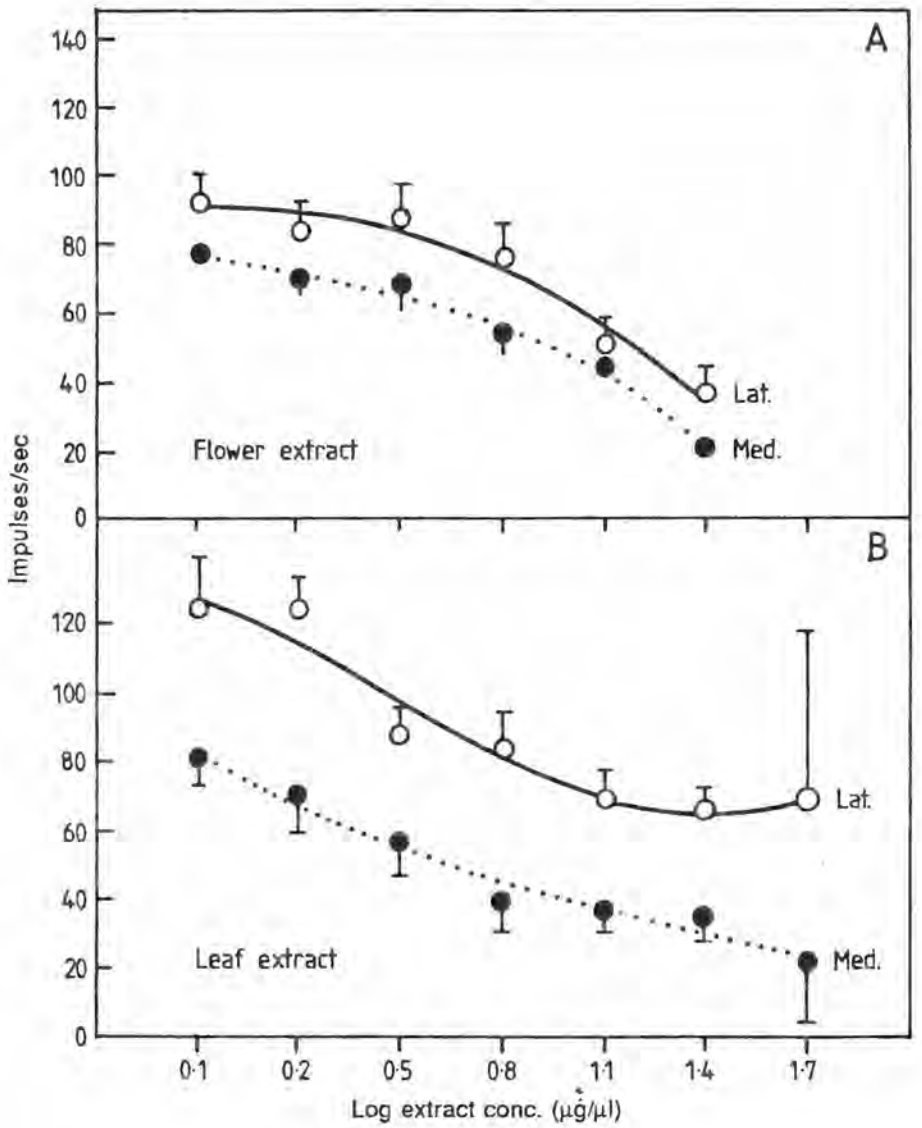


Figure 16: Electrophysiological dose response curves of gustatory cells in lateral (Lat.) and medial (Med.) styloconica sensilla of the 5th instar *M. testulalis* stimulated with aqueous extracts from cotton flowers (A) and leaves (B).

Plate 15: Spike patterns recorded from the lateral styloconicum sensillum of the fifth instar *M. testulalis* stimulated with a series of increasing concentrations of aqueous extract of cowpea flowers.

(A) 50mM NaCl

(B) 0.78ug/ul

(C) 1.5ug/ul

(D) 3.1ug/ul

(E) 6.25ug/ul

(F) 12.5ug/ul

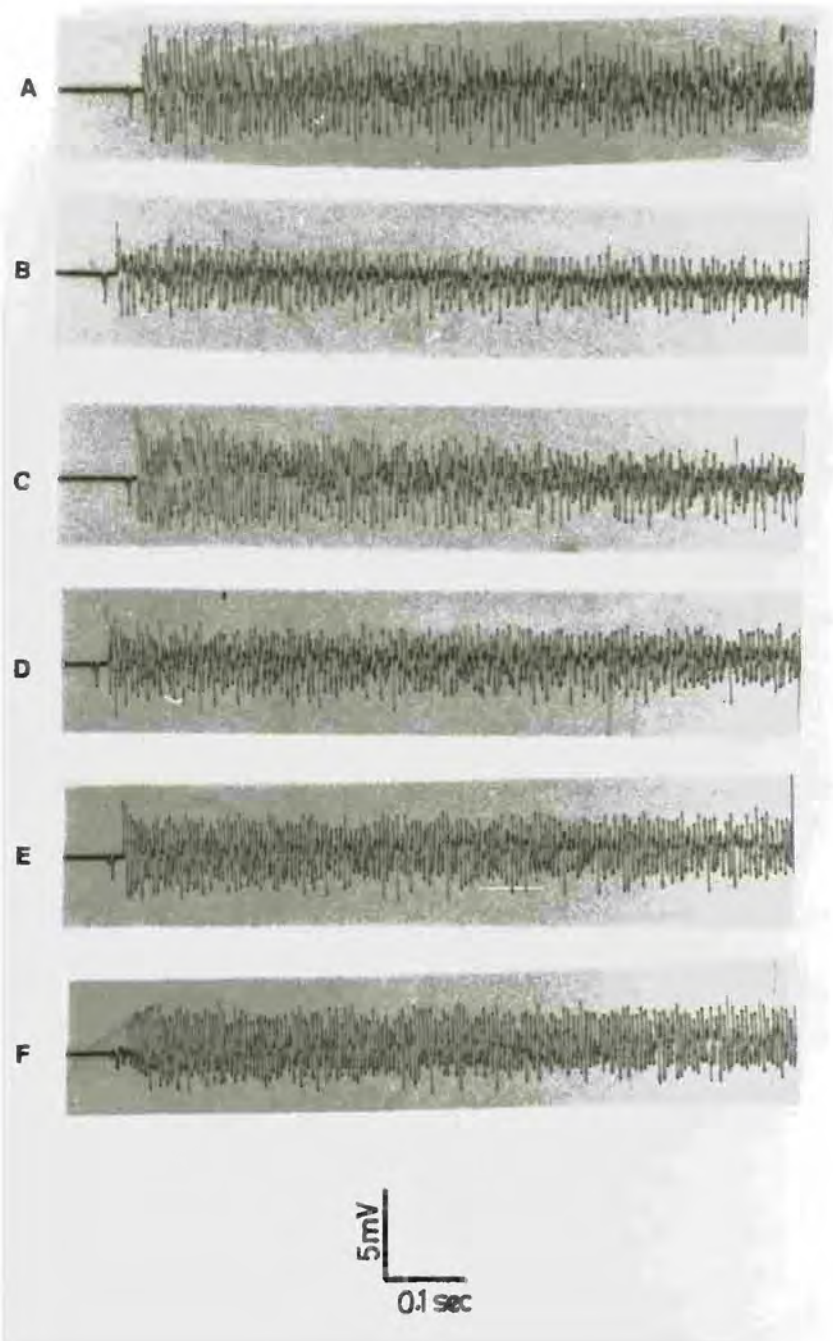

A
B
C
D
E
F
5mV
0.1 sec

Plate 16: Spike patterns recorded from the medial styloconicum sensillum of the fifth instar *M. testulalis* stimulated with a series of increasing concentrations of aqueous extract of cowpea pods.

(A) 50mM NaCl

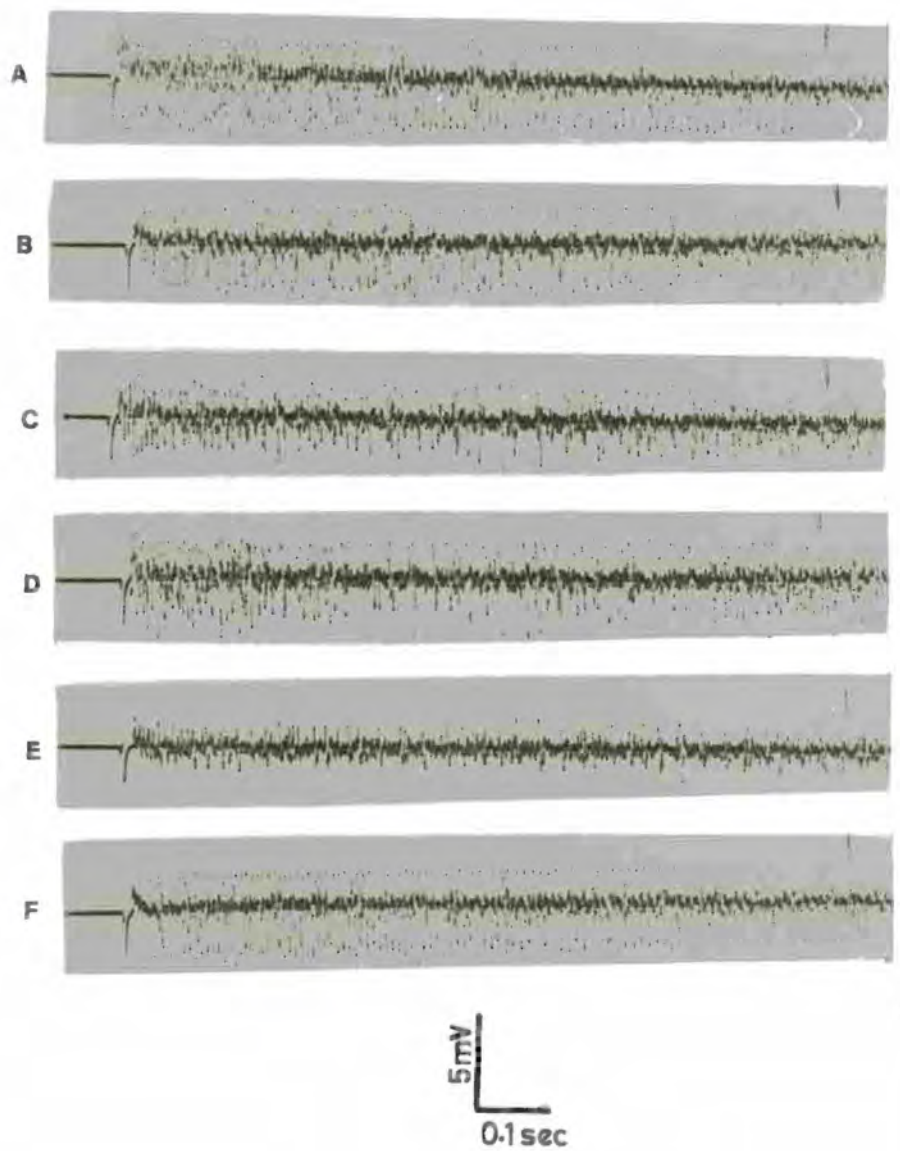
(B) 0.78ug/ul

(C) 6.25ug/ul

(D) 12.5ug/ul

(E) 50ug/ul

(F) 50mM NaCl


A
B
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5mV
0.1 sec

Plate 17: Spike patterns recorded from the lateral styloconicum sensillum of the fifth instar *M. testulalis* stimulated with a series of increasing concentrations of aqueous extract of cowpea pods.

(A) 50mM NaCl

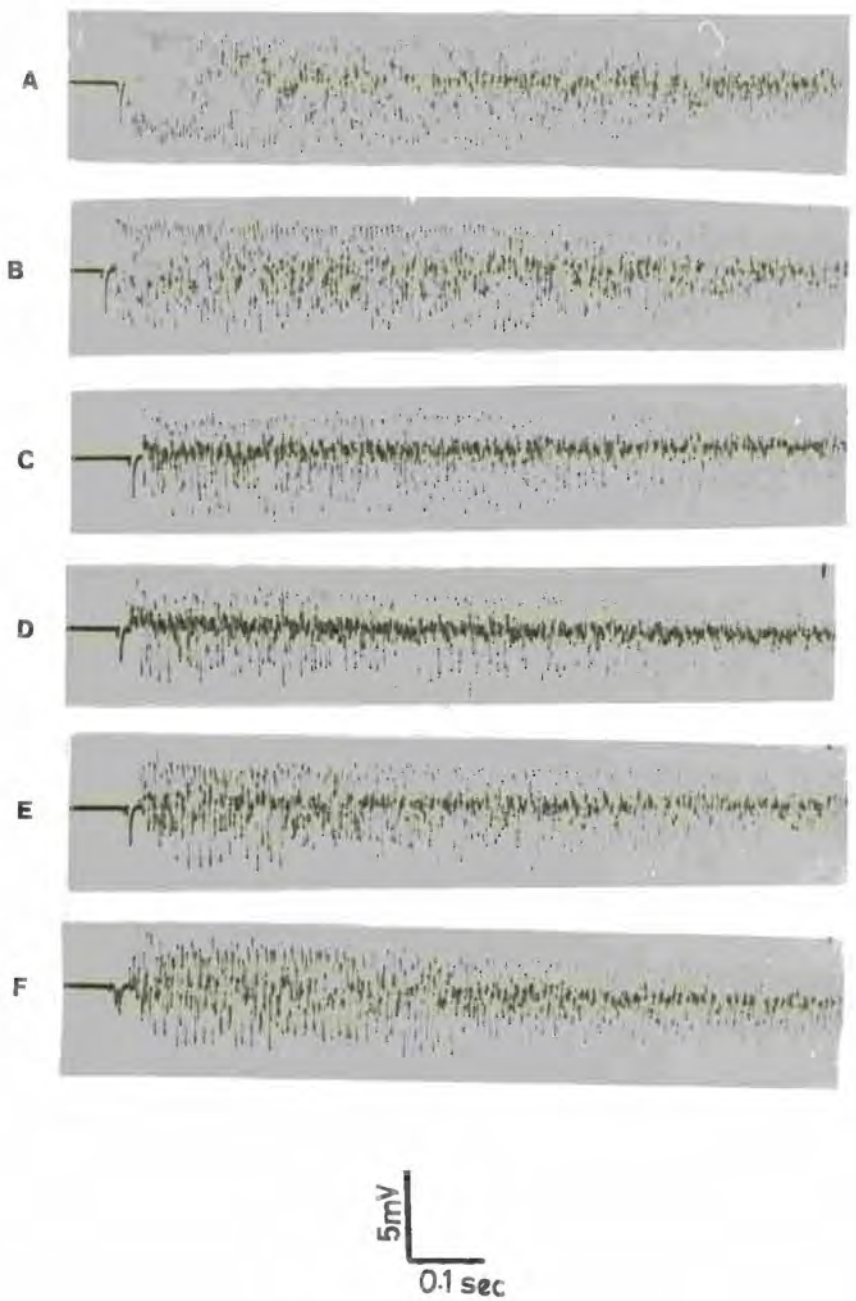
(B) 0.39ug/ul

(C) 1.5ug/ul

(D) 6.25ug/ul

(E) 25ug/ul

(F) 50mM NaCl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5mV
0.1 sec

Plate 18: Spike patterns recorded from the medial styloconicum sensillum of the fifth instar *M. testulalis* stimulated with a series of increasing concentrations of aqueous extract of cowpea leaves.

(A) 50mM NaCl

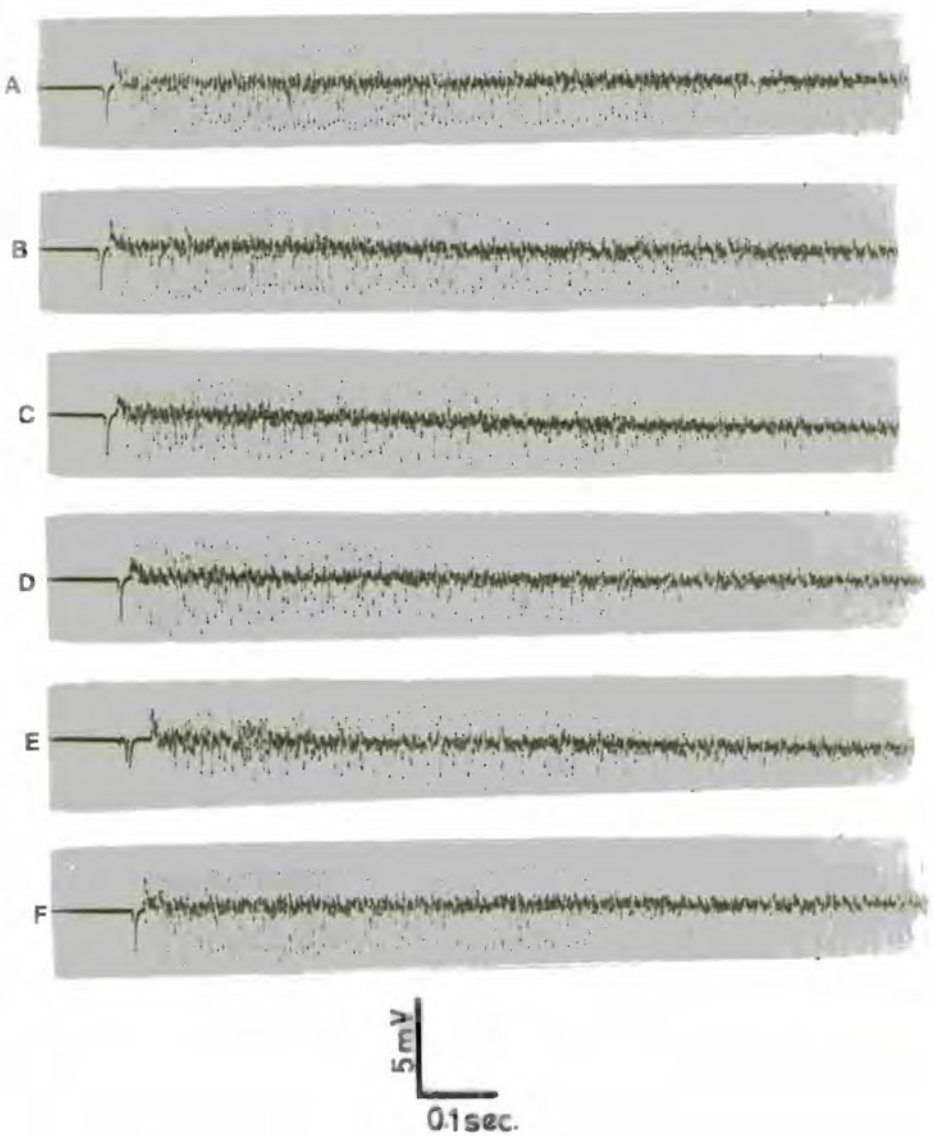
(B) 0.78ug/ul

(C) 1.5ug/ul

(D) 6.25ug/ul

(E) 25ug/ul

(F) 50mM NaCl


A
B
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F
5mV
0.1sec.

Plate 19: Spike patterns recorded from the lateral styloconicum sensillum of the fifth instar *M. testulalis* stimulated with a series of increasing concentrations of aqueous extract from common bean pods.

(A) 50mM NaCl

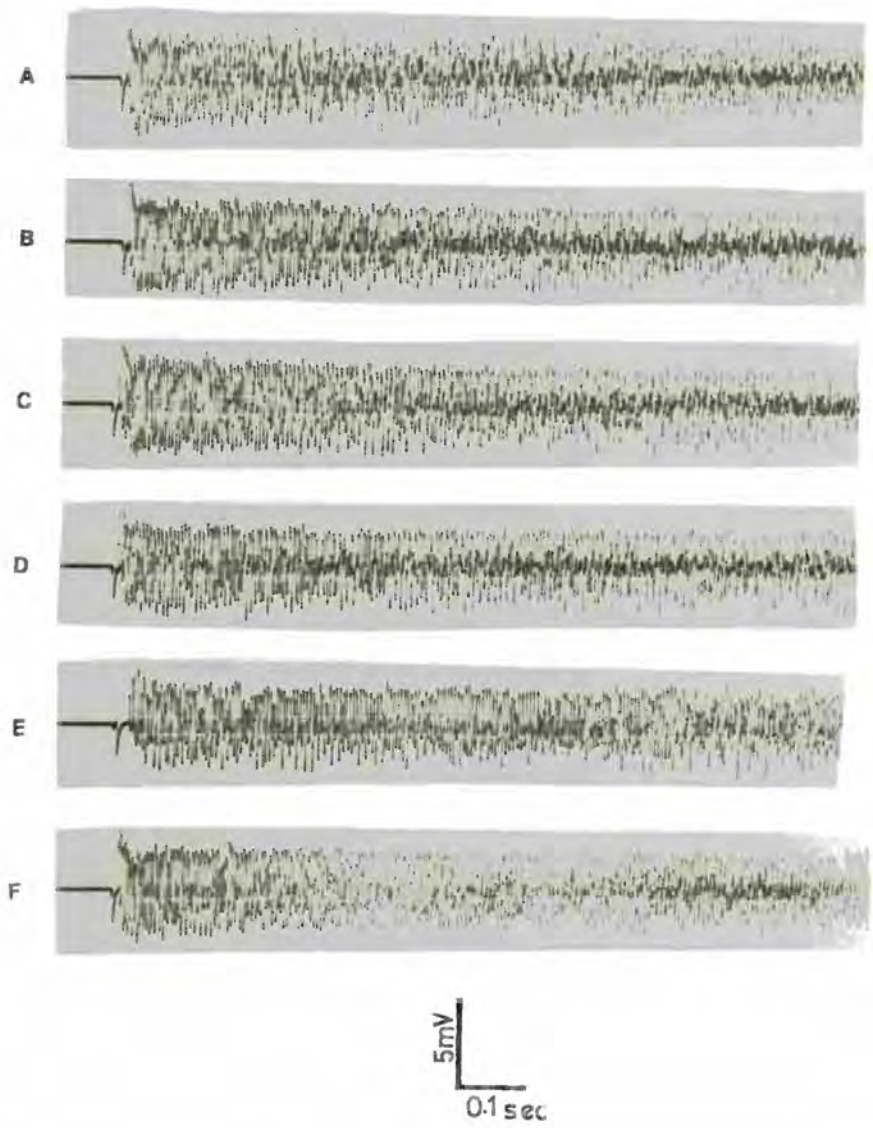
(B) 0.78ug/ul

(C) 3.1ug/ul

(D) 12.5ug/ul

(E) 50ug/ul

(F) 50mM NaCl


A
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5mV
0.1 sec

Plate 20: Spike patterns recorded from the medial styloconicum sensillum of the fifth instar *M. testulalis* stimulated with a series of increasing concentrations of aqueous extract from common bean pods.

(A) 50mM NaCl

(B) 0.78ug/ul

(C) 3.1ug/ul

(D) 12.5ug/ul

(E) 50ug/ul

(F) 50mM NaCl

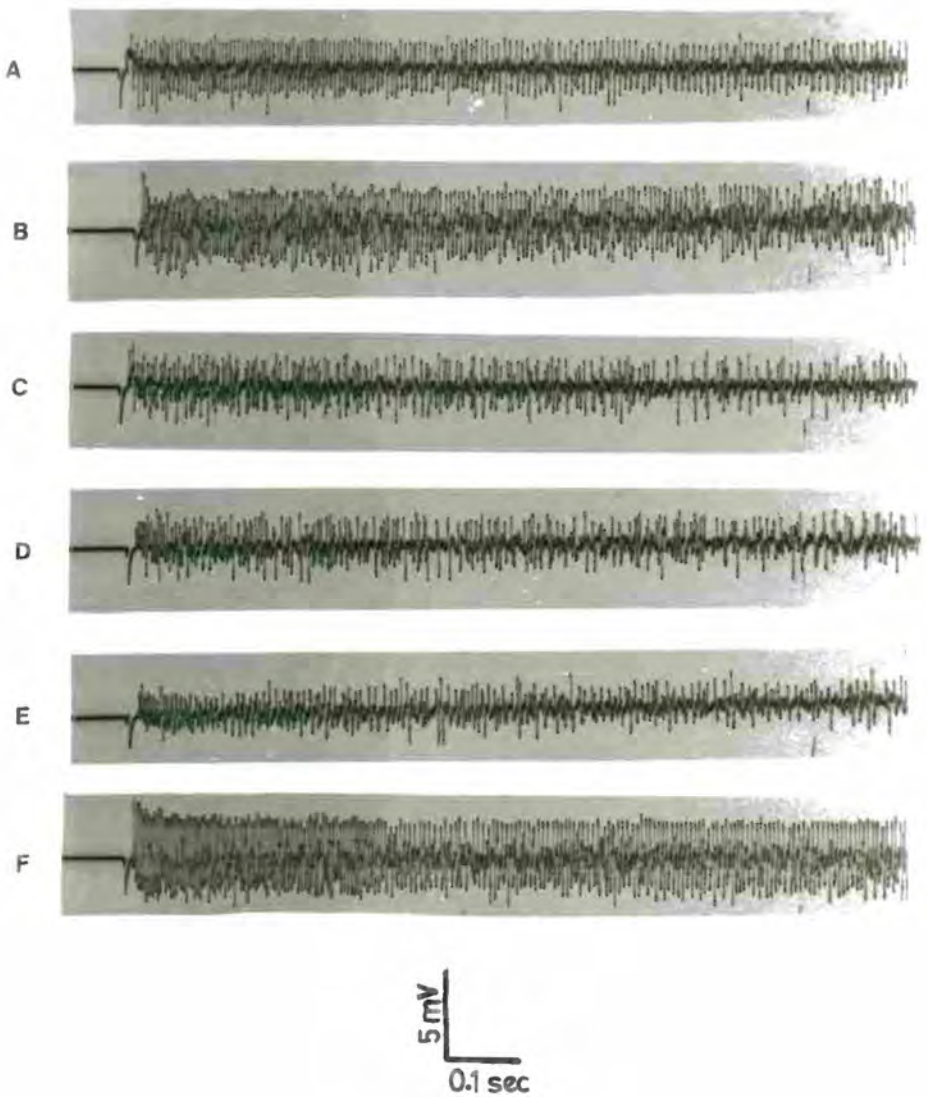

A
B
C
D
E
F
5 mV
0.1 sec

Plate 21: Spike patterns recorded from the lateral styloconicum sensillum of the fifth instar *M. testulalis* stimulated with a series of increasing concentrations of aqueous extract from common bean flowers.

(A) 50mM NaCl

(B) 0.78ug/ul

(C) 3.1ug/ul

(D) 12.5ug/ul

(E) 50ug/ul

(F) 50mM NaCl

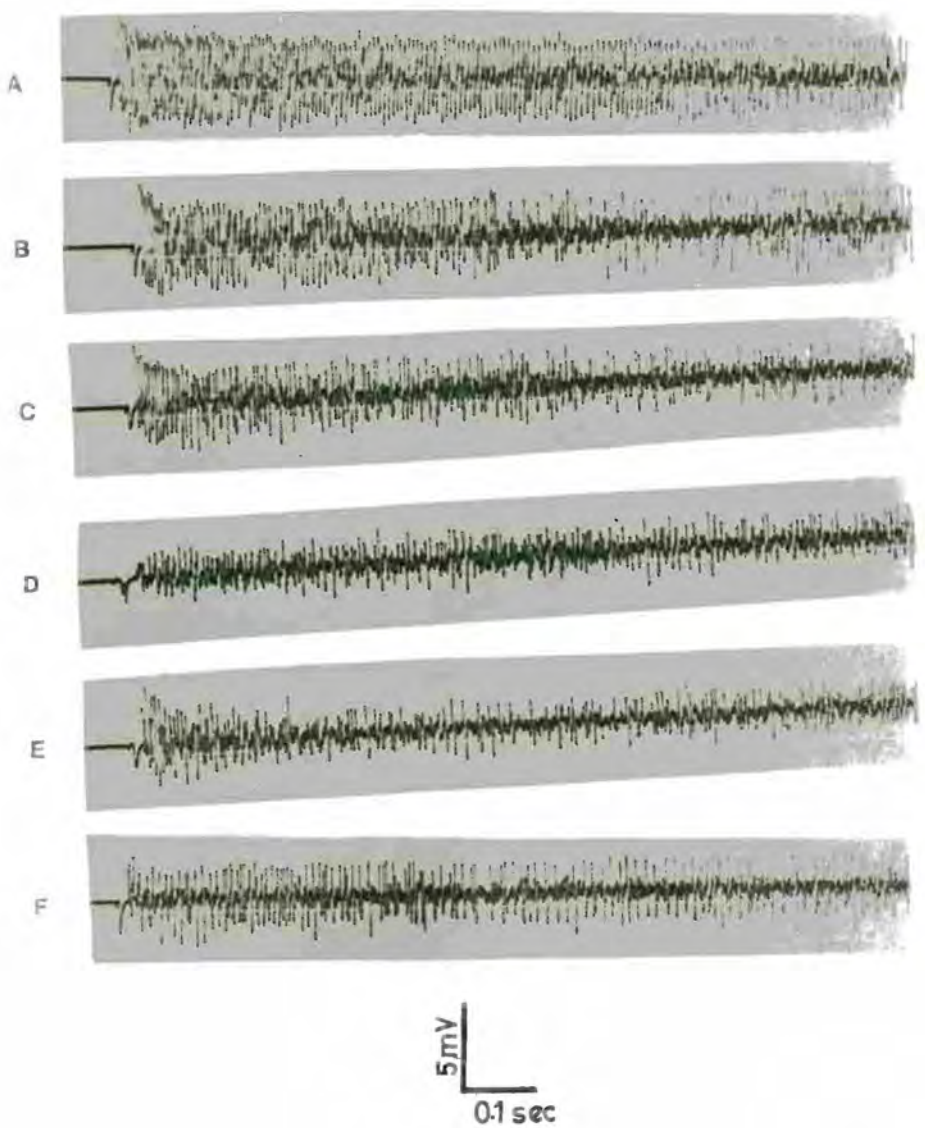

A
B
C
D
E
F
5mV
0.1 sec

Plate 22: Spike patterns recorded from the lateral styloconicum sensillum of the fifth instar *M. testulalis* stimulated with a series of increasing concentrations of aqueous extract from cotton leaves.

(A) 50mM NaCl

(B) 0.78ug/ul

(C) 3.1ug/ul

(D) 12.5ug/ul

(E) 50ug/ul

(F) 50mM NaCl

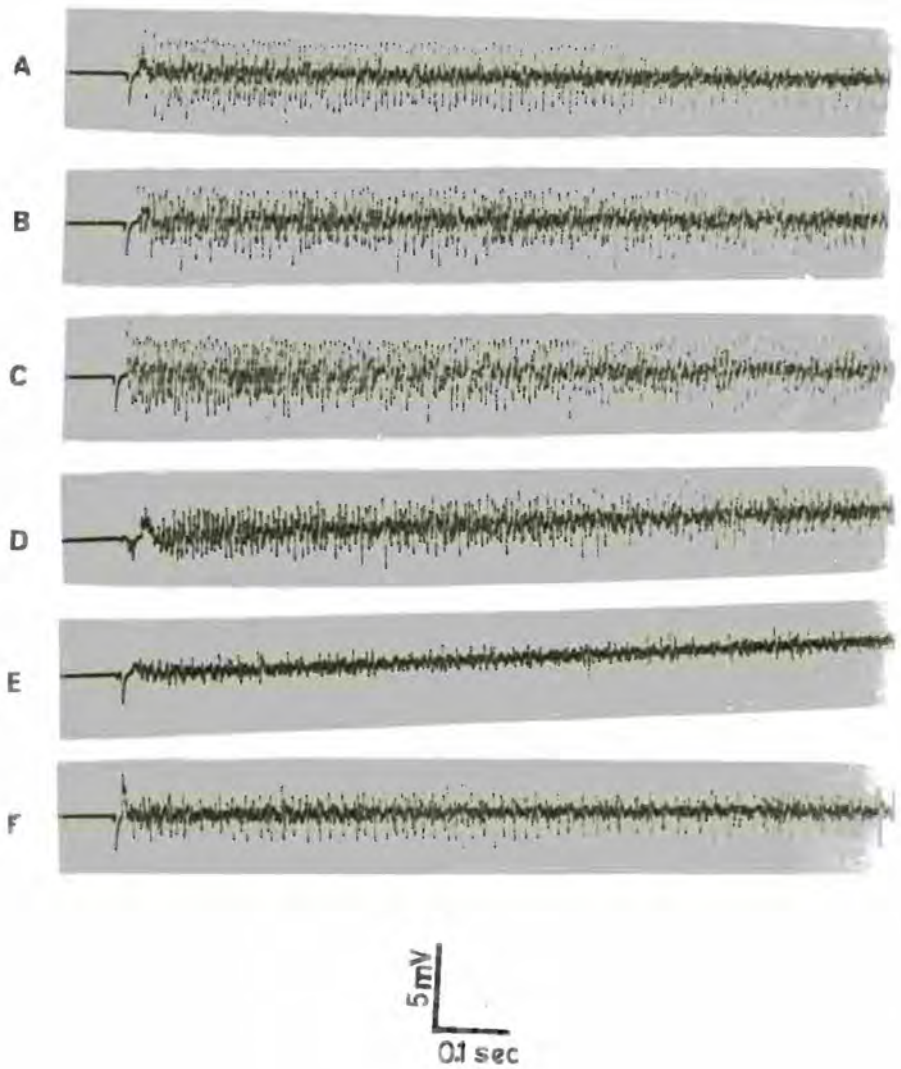

A
B
C
D
E
F
5mV
0.1 sec

Plate 23: Spike patterns recorded from the medial styloconicum sensillum of the fifth instar *M. testulalis* stimulated with a series of increasing concentrations of aqueous extract from cotton leaves.

(A) 50mM NaCl

(B) 0.78ug/ul

(C) 3.1ug/ul

(D) 6.25ug/ul

(E) 25ug/ul

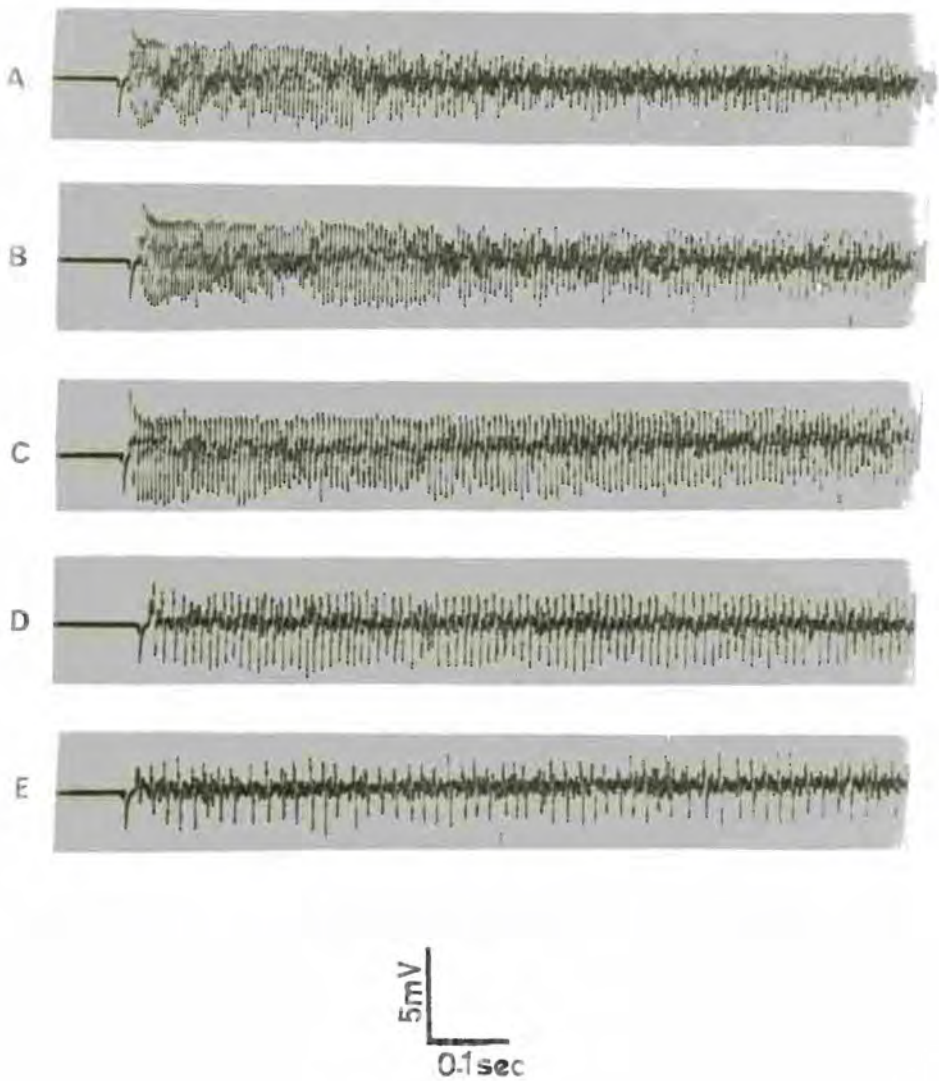

A
B
C
D
E
5mV
0.1sec

Plate 24: Spike patterns recorded from the medial styloconicum sensillum of the fifth instar *M. testulalis* stimulated with a series of increasing concentrations of aqueous extract from cotton flowers.

(A) 50mM NaCl

(B) 0.78ug/ul

(C) 1.5ug/ul

(D) 12.5ug/ul

(E) 25ug/ul

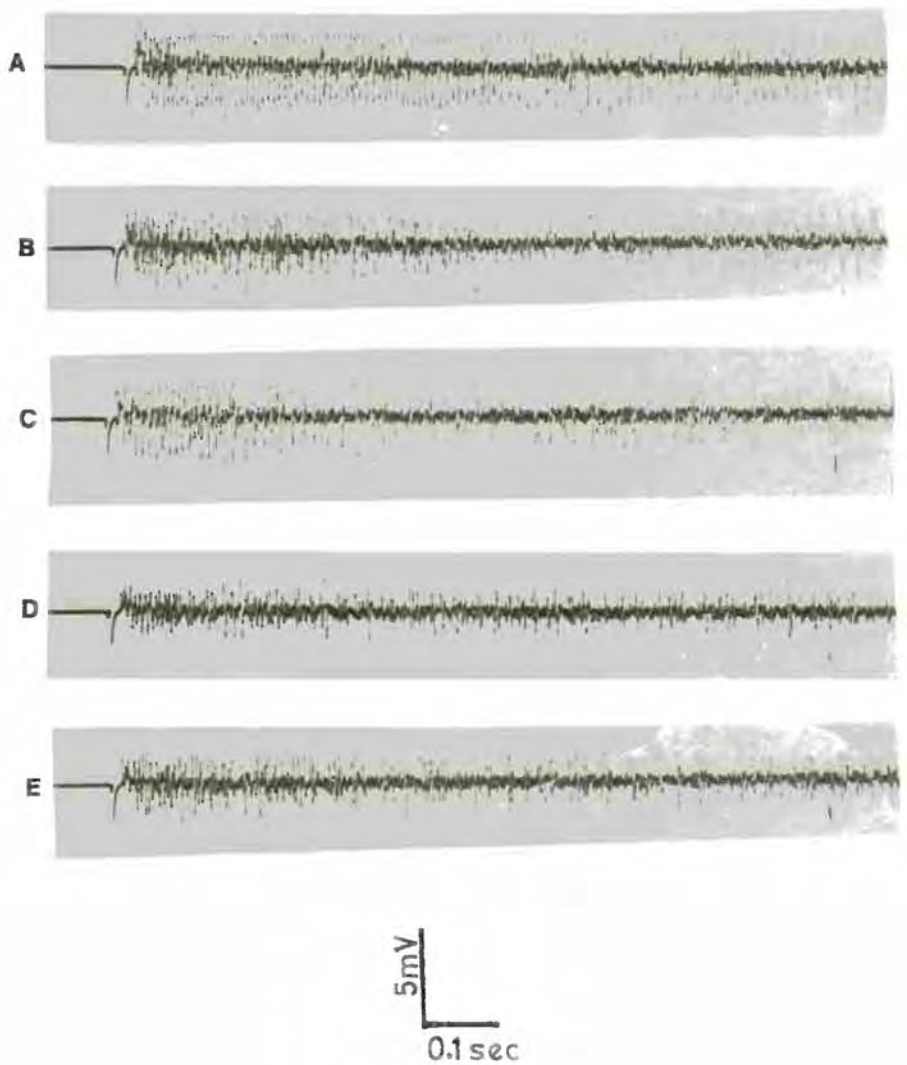

A
B
C
D
E
5mV
0.1 sec

Plate 25: Spike patterns recorded from the maxillary palp sensilla of the fifth instar *M. testulalis* stimulated with a series of increasing concentrations of aqueous extract from common bean leaves.

(A) 50mM NaCl

(B) 0.78ug/ul

(C) 1.5ug/ul

(D) 6.25ug/ul

(E) 25ug/ul

(F) 50mM NaCl

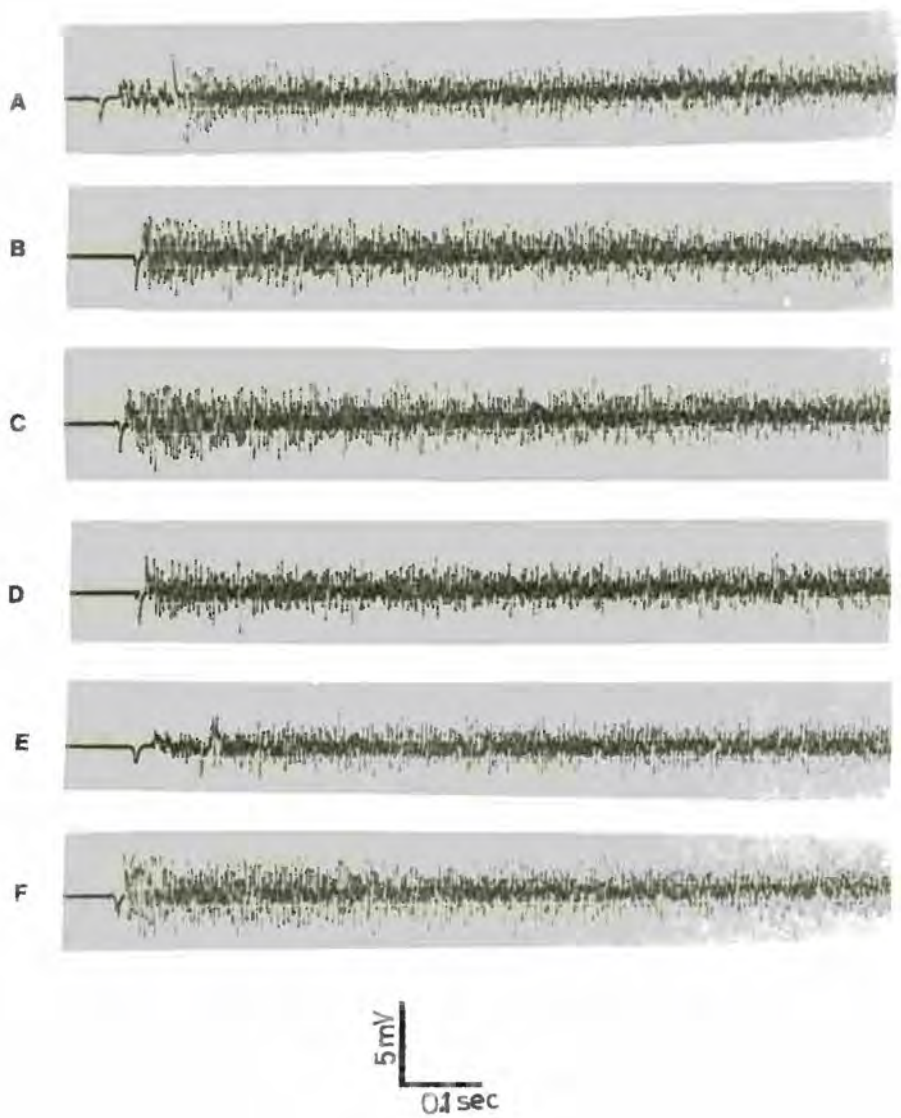

A
B
C
D
E
F
5mV
0.1 sec

Plate 26: Spike patterns recorded from the maxillary palp sensilla of the fifth instar *M. testulalis* stimulated with a series of increasing concentrations of aqueous extract from common bean flowers.

(A) 50mM NaCl

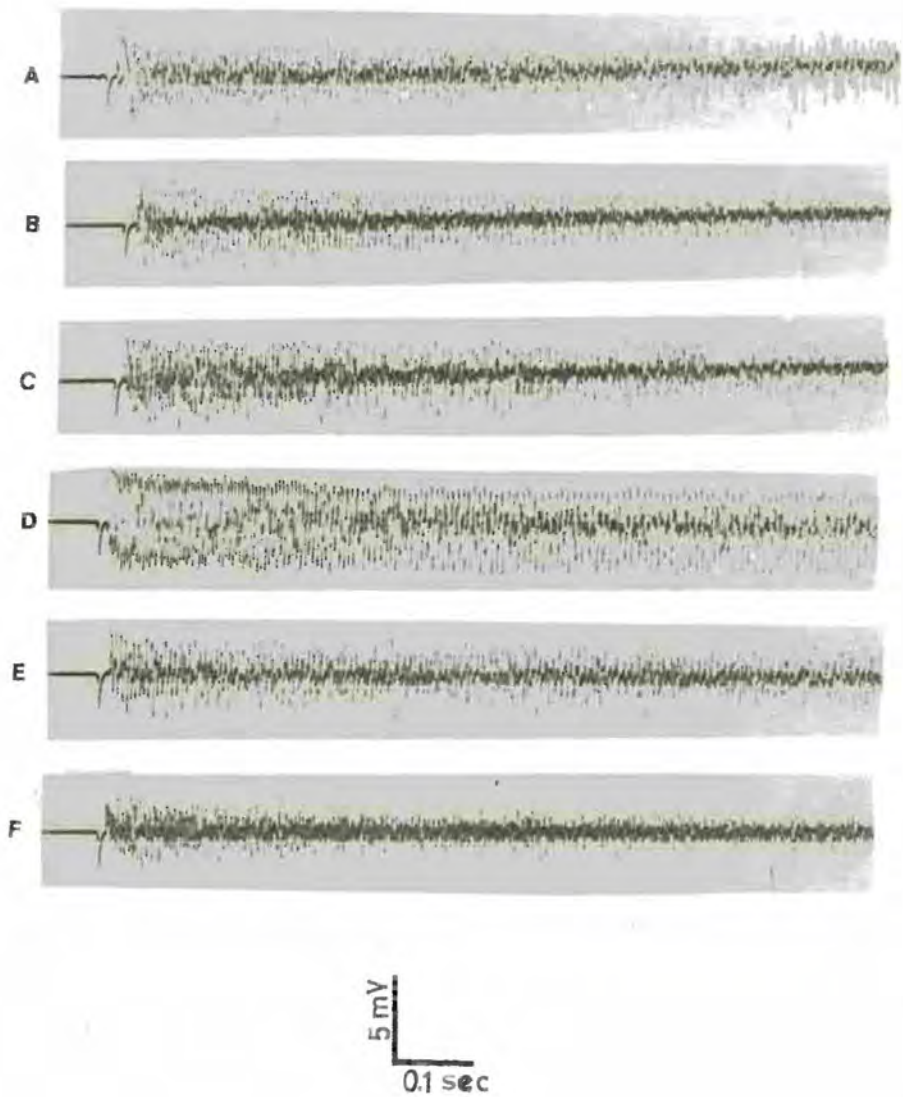
(B) 0.78ug/ul

(C) 1.5ug/ul

(D) 6.25ug/ul

(E) 25ug/ul

(F) 50mM NaCl


A
B
C
D
E
F
5 mV
0.1 sec

results showed that in order to get good results some important precautions had to be made:

1. The shape of the glass electrode tip made a very big difference in the spike pattern observed. An attempt was made to make the tip as round as possible during each recording session.

2. Animals which regurgitated after decapitation of the head were not suitable for recording. It appeared that the regurgitated fluid blocked reception of the sensillum.

3. Animals previously fed on cowpea pods that were a couple of days old gave poor recordings compared to animals fed on freshly picked pods.

Electrophysiological data showed a similar response pattern between impulse frequency by the taste cells in the lateral sensilla and the amounts ingested in bioassay (Fig. 5). This observation was a confirmation of the earlier one made by Den otter and Kahoro (1983). Sucrose is known to be a major phagostimulant among many Lepidopteran species (Thorsteinson, 1960, Schoonhoven, 1968, Bernays and Simpson, 1982). Perception of sucrose by the cells of the lateral sensilla has been reported in *Chilo*

partellus (Waladde *et al.* 1990), *Choristoneura fumiferana* (Albert, 1980, Albert and Parisella, 1988), *Pieris brassicae* (Ma, 1972) and in *Bombyx mori* and *Manduca sexta* (Ishikawa, *et al.* 1969). This suggests that the lateral sensilla in lepidopterous larvae perceives sucrose which is probably found in most host plants. On the other hand, absence of sucrose cell has been reported only in *Episema caerulocephala* (Ishikawa *et al.* 1969) which demonstrates that sucrose perception by cells of the lateral styloconica sensillum in lepidoptera is not universal.

Feeding responses to glutamine were better than methionine. However, both stimulated feeding at a concentration range between 6.25 mM - 12.5 mM. Concentrations above or below this range had negative effects to feeding. The increase in spike frequency between 6.25 - 12.5 mM in the lateral sensillum was also correlated with the behavioural bioassay results. As shown in Fig. 12 the slopes of the curves were different for each of the stimuli tested. Glutamine had a negative effect all the way while sucrose gave a totally different picture. However, as was observed for sucrose, the cells of lateral sensilla were more responsive to methionine than those of the medial sensilla which probably suggests that the sucrose sensitive cell is also sensitive to

methionine as was reported in the taste cells of the Colorado beetle, *Leptinotarsa decemlineata* (Mitchell and Harrison, 1984). Certain amino acids such as myoinosital, asparagine, adenosine possess phagostimulatory properties (Numata *et al.*, 1979; Sogawa, 1982; Hsiao, 1969) while others like alanine, serine and aminobutyric acid have been reported to inhibit feeding (Thorsteinson, 1960). Feeding response to glycine was poorer than that to the controls indicating that it is probably a feeding deterrent for *M. testulalis*.

Feeding responses to alkaloids, nicotine and tomatine, were lower than the controls . As for nicotine, an additional larvicidal property was observed suggesting that it is toxic to the larvae. The deterrent properties of nicotine and tomatine have been reported previously in several lepidopteran insects (Chapman, 1974; Ma, 1972; Brattsten, 1986) indicating that both compounds were potent antifeedants.

Feeding response of n-hexane, methanol and aqueous cowpea leaf extracts showed that aqueous extracts elicited better feeding response. Methanol elutes phospholipids while hexane elutes hydrocarbons

(Hanson, 1983). The high feeding response observed in the aqueous extracts suggests that the stimulating compounds in the cowpea were probably neither hydrocarbons nor phospholipids but were water extractable.

Stimulation of the palpal sensilla with plant extracts produced a complicated spike pattern characterised by a lot of background noise in some animals (Plate 25) and clear spike trains in others (Plate 26) depending on the sensillum stimulated. Aqueous extracts of host plant pods and flowers elicited higher feeding responses than leaves which elicited only about half the number of spikes. This high palpal sensilla inputs from pods rather than leaves was an indication that phagostimulants were higher in host plant pods and flowers than leaves. These results thus concurred with feeding bioassay results. A few studies involving recording impulses from the palp are known (eg. Den otter and Kahoro, 1983) in contrast to numerous behavioural experiments involving ablation or coating of the maxillary tip with an impermeable material as shown in several insects (Abushama, 1968; Schoonhoven, 1968; Ma, 1972; Ishikawa and Hirao, 1966; Waldbauer, 1962; Wolfenbarger *et al,* 1968). Lack of data on

electrophysiological recordings have been attributed to the multiplicity of sensilla at the palpal tip which produce a complicated response which is difficult to interpret. Scanning electron microscope revealed 5 sensilla on the maxillary palp which were probably taste receptors. Thus the variability of spike response was probably due to the fact that many sensilla were contacted by the recording glass micropipette.

The dose response curves from the lateral and medial styloconica sensilla in response to cowpea and common bean extracts was quite similar suggesting that extracts from the 2 host plants affected the taste receptor cells in the two sensilla in more or less the same way. The lateral sensilla consistently showed a higher sensitivity than the medial sensilla to the host plant extracts. Increase in the firing pattern of the lateral sensilla in response to increasing concentration of the pod and flower extracts indicated that these extracts contained a common factor that was detected by receptor cells in the lateral sensillum. Aqueous extracts contain a wide range of substances such as salts, sugars and certain amino acids which act on more than one receptor (Schoonhoven, 1986; Dethier and Crnjar, 1982). As observed in this work,

host plant extracts stimulated multineuronal responses confirming that more than one receptor was stimulated.

The poor feeding response on the cotton leaf extract correlated well with lack of feeding by the fifth instar larvae on the actual leaf suggesting that the deterrent factors were most likely to be polar compounds since they were extractable in the aqueous phase. However, the cotton flower aqueous extract surprisingly elicited a good feeding response comparable to that of the host plant flowers despite the fact that larvae did not feed on the actual flower material. This suggested that the feeding deterrent in the flower was most likely to be a non-polar compound.

The dose response curves of the lateral sensilla in response to the cotton leaf and flower extract were quite different from those of the host plant flowers and pods. This was an indication that those extracts were probably acting on different cells or on the same cells as the host plant extracts but at different receptor sites. As a result, the spike patterns and attendant behavioural responses they evoked were quite different from those evoked by host plants.

Identification of polar components in pod and flower components eliciting feeding may reveal factors that are acting on the mouthpart sensilla to stimulate

feeding and may give a further insight into feeding stimulants for *M. testulalis*. On the other hand, leaf factors from host and those of other parts of the non-host plant may elucidate the mechanism of food rejection behaviour.

CHAPTER 8

SUMMARY

1. Feeding responses of the first instar *M. testulalis* larvae on its host and non-host plant parts comprise a series of feeding bouts alternating with rest or locomotion. These responses were influenced by host, plant part, and developmental stage of the larva.

2. First instar larvae spent relatively longer time feeding on host plant flowers and pods than leaves on which they rested for longer periods. Frequency of feeding was also higher on the flowers and pods than that on leaves.

3. Long feeding bouts by first instar larvae were generally associated with long locomotory bouts. Duration of feeding bouts on cotton flowers and leaves were similar to those of cowpea but larvae spent longer rest periods on cotton leaves suugesting that continous feeding was probably being inhibited by absence of feeding stimulants or presence of feeding deterrents in cotton leaves.

4. Larval feeding on cotton leaves and flowers by first instar larvae suggested that they had a low discriminative

ability in their food selection habits as compared to the fourth instar larvae which did not feed on cotton parts at all.

5. Feeding responses of the first instar larvae on cowpea were different from those of the fourth instars. Feeding bouts in the fourth instar larvae were much shorter on leaves than on flowers and pods while rest periods were much longer on leaves. This suggested that leaf tissue may be more difficult to digest than flowers and pods.

6. Trichomes on common bean leaves are the first line of defence against *M. testulalis* larval feeding. Neither the first nor the fourth instar larvae could feed on the leaves of the common bean because of the dense trichome mat which trapped them and eventually died. Nevertheless, larvae fed very well on the flowers and pods indicating that if the larva can overcome the defensive effect of the leaves the common bean can be as susceptible as the cowpea.

7. Time of day had no significant influence on the pattern of larval feeding or quantity of food intake.

8. Duration of feeding of first instar larvae on excised leaves of cowpea was significantly reduced compared to the intact plant. It is most likely that desiccation of the excised cowpea leaves and possible enzymatic reactions may

facilitate changes in the concentrations of the chemicals in the sap. This may then alter the phagostimulatory effectiveness of the chemical components there-in. This means that observations of feeding responses on the excised leaves are not the same as those of intact parts.

9. Feeding duration of larvae on excised flowers and pods of cowpea and common bean was similar to that on intact tissues. This implied that host flowers and pods being non-photosynthetic and storage organs may possess a different set of enzymes compared to leaves. Thus, observations on feeding responses on excised flowers and pods are valid for drawing conclusions.

10. Starvation of the first instar larvae for 0, 1, 2, 3 and 4 hours did not influence feeding patterns of the larvae.

11. Pods and flowers of both host plants evoked higher feeding responses in the fourth instar larvae than any other parts of the plants in choice and no choice tests suggesting that these parts may contain higher levels of feeding stimulants.

12. Plant powder obtained by grinding air dried parts of the host plants evoked relatively low feeding responses when incorporated in agar cellulose gel as compared to fresh

plant materials. This implied that the phagostimulatory potency of the pod and flower tissues had been reduced. Powder from cotton leaves and flowers elicited reasonable feeding responses in agar cellulose gel medium suggesting that the phagodeterrency properties had been reduced by air drying. But also the texture of the agar cellulose gel versus whole plant extract is different and may also affect feeding.

13. Sucrose, glucose and fructose stimulated feeding in agar cellulose medium and cellulose acetate paper discs. Dose response curves indicated that the optimal concentrations were in the range of 0.1 - 0.2 M.

14. Methionine and glutamine stimulated feeding within a concentration range of 6.25 - 12.5 mM. A similar relationship was obtained from results of the electrophysiological bioassay.

15. Nicotine and tomatine were deterrent to larval feeding. In addition, nicotine was larvicidal at concentrations greater than 0.0045 % . However, further studies are necessary to elucidate the specificity and toxicity of this compound to other organisms in the ecosystem of *M. testulalis* larvae for them to be of potential for management strategies.

16. Aqueous extracts from host plant flowers and pods elicited feeding responses in the fourth instar larvae which were comparable to the feeding responses on the corresponding fresh parts. Extracts from leaves stimulated less feeding suggesting that optimal levels of phagostimulants were in flowers and pods.

17. Aqueous extract from cotton leaves elicited lower feeding responses suggesting that there is a water extractable substance that enhances feeding. On the other hand, aqueous extracts from cotton flowers were fed on well by the larvae in spite of the fact that larvae did not feed on the fresh flowers. This implied that any physical deterrent factor(s) were by passed by extraction while possible chemical deterrents may be insoluble in water.

18. Growth and developmental rate of the larvae were fastest on host plant flowers and pods. This suggested that in addition to having phagostimulants, these parts were the most nutritionally adequate when compared with other parts.

19. Rearing the larvae on common bean pods did not change the preference for feeding on cowpea pods by the larvae suggesting that rearing through several generations may be necessary to induce the change. However since cowpea and common bean belong to the same family, the preference for

feeding by the larvae on either of the plants may be genetically determined and not amenable to induction.

20. External morphology of the lateral and medial styloconica sensilla of the mouth parts of *M. testulalis* was similar to that described in other Lepidoptera. The sensilla on the palpal tip showed 8 hairs of which 5 were likely to be gustatory with pores at the tip. The antennae also showed 3 hairs with apical papillae at the tip similar to that of the styloconic sensilla. This suggests that they are also gustatory. Ultrastructural studies using transmission electron microscope remains to be done to determine their innervation and hence provide further evidence.

21. Aqueous extract of the host and non host plant parts as well as sucrose, NaCl, methionine and glutamine (pure compounds) elicited spike potentials in gustatory neurones of the maxillary palp, lateral and medial styloconica sensilla.

22. The palpal sensilla were sensitive to sucrose, NaCl, methionine, glutamine and aqueous extracts of host and non host plants. However, host plant extracts evoked higher impulse frequencies than non host plant extracts which were similar to the trend observed in the feeding bioassays. Thus, sensory inputs into the C.N.S from the gustatory cells in the palpal tip sensilla may complement those from

gustatory cells in the styloconica sensilla in facilitating recognition and discrimination of hosts from non-host plants.

23. The lateral styloconica sensilla were sensitive to sucrose, NaCl, and host and non-host aqueous plant extracts. Host plant pods and flowers evoked high impulse frequencies in the gustatory cells in the lateral styloconica sensillum and was similar to the pattern observed with the feeding bioassays. This indicated that they contained compounds which were "recognisable" by the C.N.S as phagostimulants. Extracts from cotton leaves evoked lower impulse frequencies than extracts from pods and flowers. This difference in taste sensilla inputs into the C.N.S appears to play a major role in the discrimination between cotton on one hand and cowpea and common beans on the other by the larvae.

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Appendix 1

Mean developmental period of larvae of *M. testulalis* reared on different parts of cowpea in the laboratory.

	Mean larval period (Days)			
	R1	R2	R3	Mean ± S.E
Leaves	17.0	18.0	18.0	17.5 ± 0.27
Flowers	12.0	12.4	13.5	12.5 ± 0.20
Pods	12.0	12.4	13.8	12.6 ± 0.18
Seed	12.0	12.0	13.4	12.5 ± 0.20

ANOVA

Source	DF	SS	MS	F	P > F
Model	5	11.697	22.3395	144.14	0.0001
Error	46	7.12915	0.154981		
Total	51	118.82			

R-Square	C.V	Root MSE	Age mean
0.94	3.04	0.3936	12.94

Source	DF	SS	MS	F	P > F
Part	3	90.098	30.0327	193.78	0.0001
Rep	2	21.599	10.7997	69.68	0.0001

Appendix 2

Mean pupal weights of *M. testulalis* larvae reared on different parts of cowpea in the laboratory.

	Pupal weight in grams			
	R1	R2	R3	Mean ± S.E
Leaves	0.026	0.030	0.017	0.025 ± 0.03
Flowers	0.049	0.044	0.045	0.046 ± 0.01
Pods	0.047	0.037	0.039	0.042 ± 0.02
Seed	0.046	0.039	0.040	0.042 ± 0.02

ANOVA

Source	DF	SS	MS	F	P > F
Model	5	0.00189	0.00037	12.83	0.0001
Error	46	0.00135	0.00002		
Total	51	0.00325			

R-Square	C.V	Root MSE	Pupal wt. mean
0.58	13.0	0.05	0.042

Source	DF	SS	MS	F	P > F
Part	3	0.00137	0.00045	15.5	0.0001
Rep.	2	0.00052	0.00026	8.83	0.006

Appendix 3

Mean developmental period of larvae of *M. testulalis* reared on different parts of common bean in the laboratory.

	Mean larval period (Days)			
	R1	R2	R3	Mean ± S.E
Leaves	19.3	-	-	19.3 ± 0.33
Flowers	11.0	12.0	13.0	12.0 ± 0.28
Pods	13.0	13.0	13.8	13.4 ± 0.13
Seed	12.0	12.5	13.0	12.5 ± 0.15

ANOVA

Source	DF	SS	MS	F	P > F
Model	5	140.717	28.1434	213.13	0.0001
Error	31	4.09344	0.13204		
Total	36	144.810			

R-Square	C.V	Root MSE	Age mean
0.9717	2.7439	0.363	13.243

Source	DF	SS	MS	F	P > F
Part	3	132.202	44.0675	333.73.	0.0001
Rep.	2	8.51478	4.2573	32.24	0.0001

Appendix 4

Mean Pupal weights of *M. testulalis* larvae reared on different parts of common bean in the laboratory.

	Pupal weight in grams			
	R1	R2	R3	X ± S.E
Leaves	0.023	-	-	0.023 ± 0.03
Flowers	0.038	0.029	0.032	0.032 ± 0.02
Pod	0.042	0.039	0.041	0.041 ± 0.01
Seed	0.039	0.033	0.033	0.036 ± 0.02

ANOVA

Source	DF	SS	MS	F	P > F
Model	5	0.00118	0.00024	10.8	0.0001
Error	31	0.00067	0.00002		
Total	36	0.00185			

R-Square	C.V	Root MSE	Pupal wt. mean
0.636	13.0	0.004	0.0357

Source	DF	SS	MS	F	P > F
Part	3	0.00094	0.00031	14.39	0.0001
Rep.	2	0.00024	0.00012	5.61	0.0083

Appendix 5

Anova for role of larval food on feeding preferences of the fifth instar *M. testulalis* larva.

Dependent variable: Dry weight pod eaten.

Source	DF	SS	MS	F-Value	P>F
Model	3	0.0228	0.00759	28.18	0.0001*
Error	86	0.02318	0.000269		
Total	89				

R-Square	C.V	Root MSE	Intake mean
0.4957	29.2	0.0164	0.056

Source	DF	SS	MS	F-Value	P>F
Rearing food	3	0.02278	0.00759	28.18	0.0001*

* Significant

Appendix 6

Feeding responses of 4th instar *M. testulalis* larvae to fructose incorporated in agar-cellulose gel.

	Quantity ingested (g. fresh wt.)			
Concentration (Molar)	R1	R2	R3	X ± S.E
1	0.093	0.012	0.083	0.097 ± 0.007
0.2	0.318	0.284	0.198	0.267 ± 0.002
0.1	0.388	0.124	0.264	0.287 ± 0.003
0.05	0.214	0.250	0.200	0.222 ± 0.002
0.01	0.026	0.024	0.016	0.022 ± 0.006
Control	0.038	0.017	0.013	0.023 ± 0.004

ANOVA

Dependent variable: Gel intake.

Source	DF	SS	MS	F	P>F
Model	7	1.1671	0.1667	38.71	0.0001
Error	97	0.4178	0.0043		
Total	104				

R-Square	C.V	Root MSE	Intake mean
0.7363	45.28	0.065	0.145

Source	DF	SS	MS	F	P>F
Conc.	5	1.1319	0.2263	52.56	0.0001
Rep.	2	0.0351	0.0175	4.08	0.0198

Appendix 7

Feeding responses of fourth instar *M. testulalis* larvae to sucrose incorporated in agar-cellulose gel.

	Quantity ingested (g. fresh wt.)			
Concentration (Molar)	R1	R2	R3	X ± S.E
1	0.090	0.092	0.086	0.091 ± 0.013
0.2	0.226	0.261	0.223	0.237 ± 0.017
0.1	0.198	0.212	0.236	0.216 ± 0.022
0.05	0.167	0.172	0.161	0.167 ± 0.015
0.01	0.112	0.079	0.064	0.083 ± 0.009
Control	0.015	0.020	0.023	0.019 ± 0.003

ANOVA

Dependent variable: Gel intake.

Source	DF	SS	MS	F	P>F
Model	7	0.8391	0.1198	17.28	0.0001
Error	151	1.0478	0.0069		
Total	158	1.8870			

R-Square	C.V	Root MSE	Intake mean
0.4447	56.24	0.083	0.148

Source	DF	SS	MS	F	P>F
Conc.	5	0.8371	0.1674	24.13	0.0001
Rep.	2	0.0020	0.0010	0.15	0.8622

Appendix 8

Feeding responses of fourth instar *M. testulalis* larvae to glucose incorporated in agar-cellulose gel.

	Quantity ingested (g. fresh wt.)			
Concentration (Molar)	Replicates R1	R2	R3	X ± S.E
1	0.177	0.133	0.186	0.165 ± 0.018
0.2	0.349	0.318	0.348	0.340 ± 0.021
0.1	0.197	0.182	0.160	0.180 ± 0.017
0.05	0.240	0.174	0.153	0.186 ± 0.015
0.01	0.050	0.052	0.050	0.051 ± 0.004
Control	0.022	0.036	0.031	0.029 ± 0.006

ANOVA

Dependent variable: Gel intake.

Source	DF	SS	MS	F	P>F
Model	7	1.6039	0.2291	30.47	0.0001
Error	157	1.1805	0.0075		
Total	164				

R-Square	C.V	Root MSE	Intake mean
0.576	50.97	0.087	0.170

Source	DF	SS	MS	F	P>F
Conc.	5	1.5845	0.3169	42.15	0.0001
Rep.	2	0.0200	0.0100	1.33	0.2667

Appendix 9

Feeding responses of the fifth instar *M. testulalis* larvae to glucose impregnated in cellulose acetate paper discs.

Concentration (mM)	Area of cellulose acetate disc eaten (mm^2 ± S.E)
12.5	19.8 ± 1.48
25	35.0 ± 3.36
50	41.9 ± 3.46
100	33.0 ± 4.64
Control	13.9 ± 3.98

Appendix 10

Feeding responses of the fifth instar *M. testulalis* larvae to sucrose impregnated in cellulose acetate paper discs.

Concentration (mM)	Area of cellulose acetate disc eaten (mm^2 ± S.E)
25	67.1 ± 8.09
50	69.0 ± 15.5
100	61.1 ± 11.0
200	79.8 ± 11.9
400	45.9 ± 7.1
Control	16.1 ± 0.96

Appendix 11

Feeding responses of the fifth instar *M. testulalis* larvae to methionine impregnated in cellulose acetate paper discs.

Concentration (mM)	Area of cellulose acetate disc eaten (mm^2 ± S.E)
6.26	22.6 ± 7.66
12.5	33.7 ± 6.58
25	3.6 ± 1.53
50	5.3 ± 2.5
100	5.8 ± 2.25
Control	11.5 ± 0.48

Appendix 12

Feeding response of the fifth instar *M. testulalis* larvae to glutamine impregnated in cellulose acetate paper discs.

Concentration (mM)	Area of cellulose acetate disc eaten (mm^2 ± S.E)
6.25	30.8 ± 4.32
12.5	39.6 ± 8.28
25	8.5 ± 2.1
50	8.4 ± 3.1
100	3.2 ± 0.96
Control	14.6 ± 1.78

Appendix 13

Feeding response of the fifth instar *M. testulalis* larvae to glycine impregnated in cellulose acetate paper discs.

Concentration (mM)	Area of cellulose acetate disc eaten (mm^2 ± S.E)
12.5	3.73 ± 0.98
25	3.06 ± 1.21
50	1.26 ± 0.44
100	1.13 ± 0.38
control	17.1 ± 0.38

Appendix 14

Feeding response of the fifth instar *M. testulalis* larvae to nicotine impregnated in cellulose acetate paper discs.

Concentration (%)	Area of cellulose acetate disc eaten (mm^2 ± S.E)
0.0045	4.60 ± 1.32
0.045	1.10 ± 0.31
0.45	5.70 ± 2.09
4.5	3.30 ± 2.05
control	11.3 ± 1.93

Appendix 15

Feeding response of the fifth instar *M. testulalis* larvae to tomatine impregnated in cellulose acetate paper discs.

Concentration (mM)	Area of cellulose acetate disc eaten (mm^2 ± S.E)
1.56	1.60 ± 0.64
3.12	2.26 ± 0.92
6.25	0.46 ± 0.23
12.5	0.4 ± 0.28
25	0.00 ± 0.00
50	0.067 ± 0.067
Control	29.3 ± 5.29

Appendix 16

Feeding response of the fifth instar *M. testulalis* larvae to n-hexane cowpea leaf extract impregnated in cellulose acetate paper discs.

Concentration (mM)	Area of cellulose acetate disc eaten (mm^2 ± S.E)
12.5	26.5 ± 5.01
25	33.8 ± 7.76
50	26.1 ± 7.53
100	58.9 ± 15.1
Control	16.8 ± 3.53

Appendix 17

Feeding response of the fifth instar *M. testulalis* larvae to methanolic cowpea leaf extract impregnated in cellulose acetate paper discs.

Concentration (mM)	Area of cellulose acetate disc eaten (mm^2 ± S.E)
6.25	33.20 ± 5.88
12.5	31.67 ± 8.99
25	35.5 ± 7.52
50	42.11 ± 6.98
control	12.83 ± 2.38

Appendix 18

Feeding response of the fifth instar *M. testulalis* larvae to aqueous cowpea pod extract impregnated in cellulose acetate paper discs.

Concentration (ug/ul)	Area of cellulose acetate disc eaten (mm^2 ± S.E)
6.25	30.1 ± 3.41
12.5	39.1 ± 4.76
25	57.4 ± 6.27
50	61.8 ± 4.42
100	53.4 ± 7.33
200	171.7 ± 16.3
Control	17.2 ± 1.06

Appendix 19

Feeding response of the fifth instar *M. testulalis* larvae to aqueous cowpea flower extract impregnated in cellulose acetate paper discs.

Concentration (ug/ul)	Area of cellulose acetate disc eaten (mm^2 ± S.E)
12.5	55.1 ± 14.2
25	76.0 ± 15.9
50	56.8 ± 11.8
100	124.6 ± 16.6
200	126.8 ± 19.4
400	106.5 ± 11.1
Control	17.0 ± 2.36

Appendix 20

Feeding response of the fifth instar *M. testulalis* larvae to aqueous common bean pod extract impregnated in cellulose acetate paper discs.

Concentration (ug/ul)	Area of cellulose acetate disc eaten (mm^2 ± S.E)
6.25	28.7 ± 2.93
12.5	51.4 ± 5.93
25	73.5 ± 8.70
50	128.6 ± 14.1
100	200.0 ± 19.9
200	228.8 ± 17.8
Control	16.3 ± 1.17

Appendix 21

Feeding response of the fifth instar *M. testulalis* larvae to aqueous common bean flower extract impregnated in cellulose acetate paper discs.

Concentration (ug/ul)	Area of cellulose acetate disc eaten (mm^2 ± S.E)
6.25	41.8 ± 6.41
12.5	38.7 ± 4.96
25	73.4 ± 11.1
50	126.3 ± 15.3
100	122.8 ± 15.7
200	141.9 ± 18.1
Control	15.9 ± 1.13

Appendix 22

Feeding response of the fifth instar *M. testulalis* larvae to aqueous cowpea leaf extract impregnated in cellulose acetate paper discs.

Concentration (ug/ul)	Area of cellulose acetate disc eaten (mm^2 ± S.E)
25	12.8 ± 1.58
50	19.5 ± 3.39
100	80.7 ± 17.56
200	68.3 ± 21.3
400	53.7 ± 14.7
Control	17.5 ± 3.36

Appendix 23

Feeding response of the fifth instar *M. testulalis* larvae to aqueous common bean leaf extract impregnated in cellulose acetate paper discs.

Concentration (ug/ul)	Area of cellulose acetate disc eaten (mm^2 ± S.E)
6.25	5.8 ± 1.62
12.5	10.5 ± 2.65
25	10.1 ± 2.97
50	20.2 ± 4.99
100	40.3 ± 6.47
200	55.8 ± 10.9
Control	14.5 ± 2.27

Appendix 24

Feeding response of the fifth instar *M. testulalis* larvae to aqueous cotton leaf extract impregnated in cellulose acetate paper discs.-

Concentration (ug/ul)	Area of cellulose acetate disc eaten (mm^2 ± S.E)
6.25	11.7 ± 4.94
12.5	7.00 ± 2.80
25	5.46 ± 3.31
50	0.60 ± 0.27
100	0.90 ± 0.33
200	0.13 ± 0.13
Control	16.5 ± 2.06

Appendix 25

Feeding response of the fifth instar *M. testulalis* larvae to aqueous cotton flower extract impregnated in cellulose acetate paper discs.

Concentration (ug/ul)	Area of cellulose acetate disc eaten (mm^2 ± S.E)
6.25	25.9 ± 5.41
12.5	28.2 ± 5.37
25	43.1 ± 6.39
50	123.5 ± 17.8
100	121.8 ± 21.5
200	141.3 ± 20.8
Control	17.5 ± 2.22

Appendix: 26

Electrophysiological responses from the maxillary palp sensilla of fifth instar *M. testulalis* larvae to NaCl.

Concentration (mM)	Spike frequency/sec Mean ± S.E	C.V
1	52.2 ± 13.3	76.5
5	54.6 ± 5.18	26.8
10	48.8 ± 10.9	66.9
25	53.2 ± 10.1	60.4
50	53.0 ± 13.6	81.6
100	71.5 ± 14.5	57.7

Regression equation (3rd Order)

$Y = 52.19 + 25.47X - 4.57X^2 + 18.8X^3$ R= 0.96

Spikes mean = 55.5

Appendix: 27

Electrophysiological responses from the lateral styloconica sensilla of fifth instar *M. testulalis* larvae to NaCl.

Concentration (mM)	Spike frequency Mean ± S.E	C.V
1	6.0 ± 3.48	164.5
5	16.5 ± 3.38	58.0
10	21.1 ± 2.98	39.9
25	23.8 ± 3.97	47.9
50	42.0 ± 5.96	40.1
100	39.6 ± 6.76	48.2

Regression equation (3rd Order)

$Y = 6.27 + 6.17X + 11.1X^2 - 2.68X^3$ R= 0.95

Spikes mean = 24.83

Appendix 28

Electrophysiological responses from the medial styloconica sensilla of fifth instar *M. testulalis* larvae to NaCl.

Concentration (mM)	Spike frequency/sec. Mean ± S.E	C.V
1	6.3 ± 2.89	137.2
5	32.0 ± 6.18	54.0
10	33.6 ± 7.99	67.2
25	40.6 ± 7.99	57.9
50	67.1 ± 15.6	66.0
100	71.5 ± 14.5	57.7

Regression equation (3rd Order)

$Y = 6.82 + 42.60X + 0.23X^2 - 9.28X^3$ R= 0.97

Spikes mean = 41.8

Appendix 29

Electrophysiological responses from the maxillary palp sensilla of fifth instar *M. testulalis* larvae to sucrose

Concentration (%)	Spike frequency/sec. Mean ± S.E	C.V
0.33	112.3 ± 9.84	25.0
0.65	113.5 ± 9.46	23.5
1.3	115.5 ± 7.45	18.4
2.6	101.6 ± 12.0	33.5
5.2	73.6 ± 11.6	44.8

Regression equation (3rd Order)

$Y = 114.9 - 4.33X + 43.7X^2 - 46.0X^3$ R= 0.99

Spikes mean = 103.3

Appendix 30

Electrophysiological responses from the lateral styloconica sensilla of fifth instar *M. testulalis* larvae to sucrose.

Concentration (%)	Spike frequency/sec. Mean ± S.E	C.V
0.33	67.5 ± 3.45	14.4
0.65	85.3 ± 10.64	35.3
1.3	97.1 ± 14.02	40.8
2.6	89.0 ± 14.14	44.9
5.2	25.0 ± 5.85	66.3

Regression equation (3rd Order)

$Y = 94.8 + 47.8X - 93.5X^2 - 157X^3$ R= 0.99

Spikes mean = 72.7

Appendix 31

Electrophysiological responses from the medial styloconica sensilla of fifth instar *M. testulalis* larvae to sucrose.

Concentration (%)	Spike frequency/sec Mean ± S.E	C.V
0.33	70.4 ± 4.86	19.55
0.65	57.1 ± 3.30	16.35
1.3	61.7 ± 7.09	32.48
2.6	51.1 ± 4.71	26.00
5.2	28.7 ± 12.2	112.57

Regression equation (3rd Order)

$Y = 58.5 + 0.046X + 5.01X^2 - 92.0X^3$ R= 0.98

Spikes mean = 53.7

Appendix 32

Electrophysiological responses from the maxillary palp sensilla of fifth instar *M. testulalis* larvae to methionine.

Concentration (mM)	Spike frequency/sec. Mean ± S.E	C.V
6.25	73.7 ± 6.77	24.3
12.5	71.0 ± 6.02	22.0
25	81.0 ± 6.93	22.6
50	75.2 ± 12.2	39.8

Regression equation (3rd Order)

$Y = 359 - 765X + 651X^2 - 175X^3$ R= 1.00

Spikes mean = 75.2

Appendix 33

Electrophysiological responses from the maxillary palp sensilla of fifth instar *M. testulalis* larvae to glutamine.

Concentration (mM)	Spike frequency/sec Mean ± S.E	C.V
6.25	85.6 ± 13.7	35.8
12.5	75.4 ± 11.2	33.3
25	97.6 ± 11.2	25.5
50	104.2 ± 14.2	30.7

Regression equation (3rd Order)

$Y = 636 - 1424X + 1157X^2 - 296X^3$ R= 1.00

Spikes mean = 90.7

Appendix 34

Electrophysiological responses from the lateral styloconica sensilla of fifth instar *M. testulalis* larvae to glutamine.

Concentration (mM)	Spike frequency/sec. Mean ± S.E)	C.V
6.25	73.5 ± 4.33	14.4
12.5	70.1 ± 8.65	30.2
25	41.6 ± 6.13	36.0
50	36.2 ± 4.56	28.2

Regression equation (3rd Order)

$Y = -406.7 + 1306X + 1121X^2 + 297X^3$ R= 1.00

Spikes mean = 55.3

Appendix 35

Electrophysiological responses from the medial styloconica sensilla of fifth instar *M. testulalis* larvae to glutamine.

Concentration (mM)	Spike frequency/sec. Mean ± S.E	C.V
6.25	71.4 ± 6.74	21.1
12.5	38.2 ± 9.29	54.4
25	27.0 ± 5.64	46.7
50	21.8 ± 4.03	41.3

Regression equation (3rd Order)

$Y = 389.1 - 693.5X + 448.2X^2 - 98.7X^3$ R= 1.00

Spikes mean = 39.6

Appendix: 36

Electrophysiological responses from the lateral styloconica sensilla of fifth instar *M. testulalis* larvae to methionine.

Concentration (mM)	Spike frequency/sec. Mean ± S.E	C.V
6.25	90.6 ± 8.47	26.44
12.5	110.9 ± 9.87	25.19
25	65.6 ± 6.24	26.92
50	61.0 ± 7.09	32.90

Regression equation (3rd Order)

$Y = -1092 + 3083X - 2529.8X^2 + 656.1X^3$ R= 1.00

Spikes mean = 82.0

Appendix: 37

Electrophysiological responses from the medial styloconica sensilla of fifth instar *M. testulalis* larvae to methionine.

Concentration (mM)	Spike frequency/sec. Mean ± S.E	C.V
6.25	80.7 ± 8.86	29.05
12.5	66.4 ± 7.73	30.82
25	62.0 ± 17.1	67.64
50	45.5 ± 2.26	12.17

Regression equation (3rd Order)

$Y = 334.5 - 632.9X + 503.1X^2 - 135.8X^3$ R= 1.00

Spikes mean = 63.6

Appendix 38

Electrophysiological responses from the maxillary palp sensilla of instar *M. testulalis* larvae to aqueous extracts of cowpea flowers.

Conc. ug/ul	Spike frequency/second X ± S.E	C.V
0.78	107.9 ± 11.23	34
1.56	98.5 ± 13.75	44
3.12	87.5 ± 14.8	53
6.25	97.2 ± 9.41	29
12.5	94.5 ± 16.27	48
25	80.1 ± 10.52	34
50	81.8 ± 19.6	63

Regression equation (3rd Order)

$Y = 103.16 - 34.60X + 37.53X^2 - 14.97X^3$ R= 0.88

P= 0.7937

Spikes mean = 93.74

Appendix 39

Electrophysiological responses from the maxillary palp sensilla of the fifth instar *M. testulalis* larvae to aqueous extracts of cowpea pods.

Conc. ug/ul	Spike frequency/second X ± S.E	C.V
0.78	135.5 ± 6.46	16
1.56	133.6 ± 8.76	22
3.12	128.7 ± 8.17	21
6.25	131.1 ± 12.65	16
12.5	126.4 ± 6.17	16
25	108.0 ± 13.3	35
50	85.6 ± 14.61	51
100	78.1 ± 12.76	43

Regression equation (3rd Order)

$Y = 134.60 + 6.67X - 0.18X^2 - 0.28X^3$ R= 0.98

P= 0.0004

Spikes mean = 119.33

Appendix 40

Electrophysiological responses from the maxillary palp sensilla of fifth instar *M. testulalis* larvae to aqueous extracts of cowpea leaves.

Conc. ug/ul	Spike frequency/second X ± S.E	C.V
0.78	88.4 ± 9.74	33
1.56	86.5 ± 8.18	28
3.12	88.2 ± 9.50	32
6.25	94.2 ± 11.53	36
12.5	102.7 ± 10.93	31
25	90.7 ± 8.6	28
50	68.5 ± 15.0	57

Regression equation (3rd Order)

$Y = 86.0 - 19.5X + 72.0X^2 - 39.3X^3$ R= 0.98

P= 0.5202

Spikes mean = 89.16

Appendix 41

Electrophysiological responses from the lateral styloconica sensilla of the fifth instar *M. testulalis* larvae to
aqueous extracts of cowpea flowers.

Conc. ug/ul	Spike frequency/second X ± S.E	C.V
0.78	101.4 ± 7.94	27
1.56	104.1 ± 7.32	24
3.12	106.1 ± 8.94	29
6.25	100.2 ± 12.3	36
12.5	108.3 ± 10.6	31
25	103.3 ± 15.87	43
50	113.4 ± 20.46	47

Regression equation (3rd Order)

$Y = 102.89 + 11.70X - 21.80X^2 + 10.78X^3$ R= 0.80

P= 0.9912

Spikes mean = 104.94

Appendix 42

Electrophysiological responses from the lateral styloconica sensilla of the fifth instar *M. testulalis* larvae to aqueous extracts of cowpea pods.

Conc. ug/ul	Spike frequency/second X ± S.E	C.V
0.78	123.6 ± 12.71	34
1.56	131.3 ± 7.89	19
3.12	130.8 ± 10.39	26
6.25	135.5 ± 11.31	27
12.5	141.4 ± 9.86	23
25	130.2 ± 13.22	30
50	121.4 ± 14.91	32
100	109.7 ± 13.15	33

Regression equation (3rd Order)

$Y = 125.8 + 18.87X - 2.38X^2 - 5.67X^3$ R= 0.96

P= 0.7116

Spikes mean = 129.00

Appendix 43

Electrophysiological responses from the lateral styloconica sensilla of fifth instar *M. testulalis* larvae to aqueous extracts of cowpea leaves.

Conc. ug/ul	Spike frequency/second X ± S.E	C.V
0.78	102.2 ± 8.35	24
1.56	115.3 ± 14.55	37
3.12	97.9 ± 11.33	34
6.25	90.8 ± 7.41	23
12.5	87.7 ± 12.80	43
25	94.1 ± 13.22	42
50	55.0 ± 23.79	74

Regression equation (3rd Order)

$Y = 105.4 - 15.4X + 20.8X^2 - 16.07X^3$ R= 0.88

P= 0.2731

Spikes mean = 95.85

Appendix 44

Electrophysiological responses from the medial styloconica sensilla of fifth instar *M. testulalis* larvae to aqueous extract of cowpea leaves.

Conc. ug/ul	Spike frequency/second X ± S.E	C.V
0.78	95.3 ± 6.59	20
1.56	93.8 ± 7.69	24
3.12	82.1 ± 8.67	31
6.25	86.5 ± 5.19	18
12.5	75.8 ± 9.39	37
25	67.8 ± 9.0	37
50	72.3 ± 12.37	48

Regression equation (3rd Order)

$Y = 94.61 - 6.98X + 20.1X^2 + 9.43X^3$ R= 0.94

P= 0.1953

Spikes mean = 82.39

Appendix 45

Electrophysiological responses from the medial styloconica sensilla of the fifth instar *M. testulalis* larvae to aqueous extract of cowpea flowers.

Conc. ug/ul	Spike frequency/second X ± S.E	C.V
0.78	92.3 ± 8.58	32
1.56	83.6 ± 6.34	26
3.12	90.0 ± 6.62	24
6.25	82.6 ± 9.4	35
12.5	80.4 ± 6.4	25
25	73.1 ± 10.69	41
50	70.4 ± 5.91	18

Regression equation (3rd Order)

$Y = 89.80 - 7.86X + 0.68X^2 - 1.83X^3$ R= 0.94

P= 0.5385

Spikes mean = 83.36

Appendix 46

Electrophysiological responses from the medial styloconica sensilla of the fifth instar *M. testulalis* larvae to aqueous extracts of cowpea pods.

Conc. ug/ul	Spike frequency/second X ± S.E	C.V
0.78	111.0 ± 7.73	24
1.56	106.6 ± 10.0	31
3.12	107.9 ± 7.37	22
6.25	109.0 ± 9.94	31
12.5	95.0 ± 8.50	29
25	90.0 ± 5.56	18
50	92.0 ± 15.39	47
100	85.3 ± 8.65	28

Regression equation (3rd Order)

$Y = 110.16 + 2.73X - 0.19X^2 + 5.58X^3$ R= 0.94

P= 0.3641

Spikes mean = 100.91

Appendix 47

Electrophysiological responses from the maxillary palp sensilla of the fifth instar *M. testulalis* larvae to aqueous extracts of common bean pods.

Conc. ug/ul	Spike frequency/second X ± S.E	C.V
0.78	77.3 ± 12.64	56
1.56	97.6 ± 14.82	52
3.12	79.0 ± 12.89	56
6.25	82.1 ± 15.66	66
12.5	71.5 ± 14.5	70
25	44.9 ± 9.83	72
50	37.2 ± 13.56	109

Regression equation (3rd Order)

$Y = 84.62 + 41.47X - 75.94X^2 + 20.40X^3$ R= 0.96

P= 0.0466

Spikes mean = 71.52

Appendix 48

Electrophysiological responses from the maxillary palp sensilla of the fifth instar *M. testulalis* larvae to stimulation with different concentrations of aqueous extracts of common bean flowers.

Conc. ug/ul	Spike frequency/second X ± S.E	C.V
0.78	91.6 ± 6.77	26
1.56	86.6 ± 6.61	27
3.12	84.6 ± 8.68	36
6.25	54.0 ± 8.75	49
12.5	86.4 ± 5.77	21
25	59.9 ± 7.61	40
50	64.6 ± 10.28	50
100	29.5 ± 29.5	141

Regression equation (3rd Order)

$Y = 89.28 - 16.10X - 24.62X^2 + 10.45X^3$ R= 0.91

P= 0.0004

Spikes mean = 67.121

Appendix 49

Electrophysiological responses from the maxillary palp sensilla of the fifth instar *M. testulalis* larvae to aqueous extracts of common bean leaves.

Conc. ug/ul	Spike frequency/second X ± S.E	C.V
0.78	84.3 ± 6.17	23
1.56	77.8 ± 7.53	30
3.12	67.1 ± 3.53	16
6.25	66.2 ± 4.43	20
12.5	60.6 ± 6.70	33
25	45.6 ± 5.57	36
50	46.4 ± 7.96	45

Regression equation (3rd Order)

$Y = 81.61 - 22.05X - 1.15X^2 + 0.79X^3$ R= 0.98

P= 0.0001

Spikes mean = 65.16

Appendix 50

Electrophysiological responses from the lateral styloconica sensilla of the fifth instar *M. testulalis* larvae to aqueous extracts of common bean pods.

Conc. ug/ul	Spike frequency/second X ± S.E	C.V
0.78	114.2 ± 15.0	45
1.56	112.5 ± 10.14	31
3.12	118.7 ± 12.25	35
6.25	135.2 ± 13.86	35
12.5	113.7 ± 12.86	39
25	104.0 ± 16.53	52
50	78.2 ± 18.17	69

Regression equation (3rd Order)

$Y = 113.37 + 13.29X + 18.43X^2 - 22.90X^3$ R= 0.94

P= 0.2513

Spikes mean = 112.28

Appendix 51

Electrophysiological responses from the lateral styloconica sensilla of the fifth instar *M. testulalis* larvae to aqueous extracts of common bean flowers.

Conc. ug/ul	Spike frequency/second X ± S.E	C.V
0.78	99.1 ± 9.59	34
1.56	91.8 ± 8.35	35
3.12	93.7 ± 7.78	31
6.25	101.2 ± 12.57	44
12.5	103.0 ± 12.33	43
25	92.4 ± 10.72	40
50	89.6 ± 14.92	57
100	22.5 ± 12.78	113

Regression equation (3rd Order)

$Y = 94.73 - 42.87X + 101.01X^2 - 48.23X^3$ R= 0.97

P= 0.0627

Spikes mean = 92.092

Appendix 52

Electrophysiological responses from the medial styloconica sensilla of the fifth instar *M. testulalis* larvae to aqueous extracts of common bean pods.

Conc. ug/ul	Spike frequency/second X ± S.E	C.V
0.78	98.3 ± 7.55	26
1.56	98.3 ± 7.47	26
3.12	110.2 ± 10.0	31
6.25	94.1 ± 9.6	35
12.5	72.5 ± 12.76	60
25	41.9 ± 8.35	63
50	25.0 ± 10.38	117

Regression equation (3rd Order)

$Y = 101.24 + 47.12X - 90.86X^2 - 21.12X^3$ R= 0.99

P= 0.0001

Spikes mean = 80.79

Appendix 53

Electrophysiological responses from the medial styloconica sensilla of the fifth instar *M. testulalis* larvae to aqueous extracts of common bean flowers.

Conc. ug/ul	Spike frequency/second X ± S.E	C.V
0.78	90.5 ± 13.56	56
1.56	94.0 ± 12.15	48
3.12	88.0 ± 11.77	50
6.25	80.6 ± 11.13	49
12.5	89.0 ± 9.49	35
25	76.1 ± 9.81	42
50	50.9 ± 10.98	71
100	8.5 ± 1.5	24

Regression equation (3rd Order)

$Y = 90.56 - 16.82X + 35.89X^2 - 23.09X^3$ R= 0.95

P= 0.0447

Spikes mean = 80.66

Appendix 54

Electrophysiological responses from the lateral styloconica sensilla of the fifth instar *M. testulalis* larvae to extracts of aqueous common bean leaves.

Conc. ug/ul	Spike frequency/second X ± S.E	C.V
0.78	107.2 ± 6.94	20
1.56	96.3 ± 7.87	24
3.12	98.1 ± 9.79	33
6.25	88.2 ± 11.8	40
12.5	53.0 ± 5.0	28
25	66.6 ± 8.0	36
50	48.8 ± 11.0	63

Regression equation (3rd Order)

$Y = 105.23 - 6.64X - 43.20X^2 + 16.62X^3$ R= 0.93

P= 0.0001

Spikes mean = 81.24

Appendix 55

Electrophysiological responses from the medial styloconica sensilla of the fifth instar *M. testulalis* larvae to aqueous extracts of common bean leaves.

Conc. ug/ul	Spike frequency/second X ± S.E	C.V
0.78	92.8 ± 13.52	46
1.56	79.3 ± 8.26	32
3.12	80.4 ± 8.48	33
6.25	69.6 ± 6.32	27
12.5	51.6 ± 6.28	36
25	47.6 ± 4.23	26
50	42.6 ± 8.86	62

Regression equation (3rd Order)

$Y = 89.28 - 16.10X - 24.62X^2 + 10.45X^3$ R= 0.91

P= 0.0004

Spikes mean = 67.121

Appendix 56

Electrophysiological responses from the maxillary palp sensilla of the fifth instar *M. testulalis* larvae to aqueous extracts of cotton leaves.

Conc. ug/ul	Spike frequency/second X ± S.E	C.V
0.78	69.9 ± 4.98	25
1.56	72.7 ± 8.58	42
3.12	56.0 ± 7.10	45
6.25	52.8 ± 6.27	41
12.5	52.4 8.14	53
25	42.7 ± 8.0	65

Regression equation (3rd Order)

$Y = 70.24 - 14.63X - 11.79X^2 + 6.37X^3$ R= 0.94

P= 0.004

Spikes mean = 58.13

Appendix 57

Electrophysiological responses from the maxillary palp sensilla of fifth instar *M. testulalis* larvae to aqueous extracts of cotton flowers.

Conc. ug/ul	Spike frequency/second X ± S.E	C.V
0.78	71.6 ± 7.52	31
1.56	61.3 ± 4.96	28
3.12	50.4 ± 6.74	46
6.25	45.1 ± 5.1	39
12.5	30.3 ± 4.0	42
25	27.4 ± 9.0	104

Regression equation (3rd Order)

$Y = 68.01 - 31.15X - 6.96X^2 - 5.77X^3$ R= 0.99

P= 0.0001

Spikes mean = 48.82

Appendix 58

Electrophysiological responses from the lateral styloconica sensilla of the fifth instar *M. testulalis* larvae to aqueous extracts of cotton leaves.

Conc. ug/ul	Spike frequency/second X ± S.E	C.V
0.78	124.4 ± 15.54	45
1.56	124.4 ± 9.93	28
3.12	88.5 ± 8.77	35
6.25	85.1 ± 11.15	45
12.5	70.7 ± 8.73	42
25	67.8 ± 7.0	35
50	71.0 ± 49.0	97

Regression equation (3rd Order)

$Y = 123.40 - 40.78X - 30.02X^2 + 21.32X^3$ R= 0.97

P= 0.0005

Spikes mean = 93.68

Appendix 59

Electrophysiological responses from the medial styloconica sensilla of the fifth instar *M. testulalis* larvae to aqueous extracts of cotton leaves.

Conc. ug/ul	Spike frequency/second X ± S.E	C.V
0.78	81.4 ± 7.88	34
1.56	71.2 ± 10.97	55
3.12	57.5 ± 9.34	58
6.25	40.2 ± 7.853	67
12.5	38.4 ± 6.213	56
25	36.6 ± 6.6	57
50	23.5 ± 17.5	105

Regression equation (3rd Order)

$Y = 77.23 - 52.35X - 20.19X^2 + 4.28X^3$ R= 0.98

P= 0.0005

Spikes mean = 54.077

Appendix 60

Electrophysiological responses from the medial styloconica sensilla of the fifth instar *M. testulalis* larvae to aqueous extracts of cotton flowers.

Conc. ug/ul	Spike frequency/second X ± S.E	C.V
0.78	77.4 ± 3.54	16
1.56	70.6 ± 4.88	23
3.12	69.1 ± 7.33	36
6.25	55.2 ± 5.64	33
12.5	45.6 ± 3.73	25
25	21.9 ± 3.98	57

Regression equation (3rd Order)

$Y = 75.13 - 17.64X + 7.80X^2 - 15.93X^3$ R= 0.99

P= 0.0001

Spikes mean = 58.05

Appendix 61

Electrophysiological responses from the lateral styloconica sensilla of the fifth instar *M. testulalis* larvae to aqueous extracts of cotton flowers.

Conc. ug/ul	Spike frequency/second X ± S.E	C.V
0.78	92.1 ± 8.48	31
1.56	84.2 ± 8.85	36
3.12	88.1 ± 10.48	41
6.25	77.1 ± 10.10	45
12.5	52.4 ± 7.82	47
25	37.7 ± 8.73	73

Regression equation (3rd Order)

$Y = 90.60 + 0.321X - 25.58X^2 - 1.87X^3$ R= 0.98

P= 0.0004

Spikes mean = 73.55