OVIPOSITION BEHAVIOUR OF MARUCA TESTULALIS [GEYER] [LEPIDOPTERA: PYRALIDAE] WITH RESPECT TO HOST-PLANT RECOGNITION.

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THIS THESIS IS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE DOCTOR OF PHILOSOPHY DEGREE IN APPLIED ENTOMOLOGY OF THE RIVERS STATE UNIVERSITY OF SCIENCE AND TECHNOLOGY, PORT HARCOURT.

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DECLARATION

THIS IS TO CERTIFY THAT THE WORK REPORTED HERE IS MY OWN ORIGINAL STUDIES AND ALL HELP ARE DULY ACKNOWLEDGED; AND IT HAS NOT BEEN SUBMITTED TO THIS OR ANY OTHER UNIVERSITY FOR ANY OTHER DEGREE BEFORE

Dorcas D. S. Bawo

DEDICATION

THIS WORK IS DEDICATED TO THE ALMIGHTY, THE ONLY TRUE GOD, TO MY LORD AND SAVIOUR JESUS CHRIST AND TO GOD'S MOST HOLY SPIRIT WHO GUIDED ME SAFELY THROUGH ALL THE TRIALS AND TEMPTATIONS DURING THE COURSE OF THIS WORK IN KENYA.

CERTIFICATION

WE CERTIFY THAT THIS WORK WAS CARRIED OUT BY

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ABSTRACT

The oviposition behaviour of *Maruca testulalis* on host plant (cowpea), non-host plants (cotton and sunhemp), and artificial substrates (filter, butter, crepe, and aluminium foil papers) was observed in laboratory and field. These studies were carried out with the aim of gathering data on oviposition behaviour patterns especially on how the ovipositing female interacts with host plants, non-host plants as well as potential artificial oviposition substrates. It was also intended to generate information about the sensory structures involved in this biological activity.

Oviposition activities commenced two hours after lights-off increasing to a peak, four hours later. Gravid moths laid eggs on all the plant substrates tested and the preferences were as follows : cowpea > cotton > sunhemp. Among the artificial substrates examined, crepe paper evoked the best oviposition response in choice and non-choice experiments. These results suggest that physical texture (mechanical stimuli) of the oviposition substrate can influence choice of oviposition site in *M. testulalis*.

During the oviposition process, a gravid female

displayed several behavioural manoeuvres which included antennae waving, feeding, flight, walking, abdomen bending, pulsating of the terminal abdominal segments, wing twitching, ovipositor tip dragging, and searching with ovipositor tip before egg deposition. These events occurred in various sequences. The deposition of eggs at any one position was immediately followed by flight. The ovipositing moth always chose a new area. Three bouts of oviposition were observed within the six hours observation periods. Most of these activities occurred in the early part of the dark period. Females were generally quiescent in the latter part of the dark period. The pattern of egg distribution within the experimental cages and on the leaf was contagious with a negative binomial distribution. The eggs were laid singly or in loose clusters (2-16 eggs per cluster). The antennae and especially the ovipositor are the main parts of the body involved in locating the site for egg deposition.

Light and electron microscopy studies revealed that these structures i.e the antennae and ovipositor are equipped with various types of sensilla. Five main sensilla types including sensilla trichodea, basiconica and coeloconica were found on the female antennae while two main types of sensilla (trichoid chemo mechano-sensilla) were found on the ovipositor tip.

Host plant odour, texture of oviposition substrates and components in the plant extracts were found to influence oviposition response of *M. testulalis*. Cowpea extracts evoked better oviposition responses than cotton and sunhemp extracts. These studies have created a basis for further investigations on *M. testulalis* oviposition behaviour.

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KEY TO ABBREVIATIONS

ANOVA.....Analysis of variance

Corr.tt.....Corrected total

C.V.....Coefficient of variation

DF.....Degrees of freedom

E.A.H.....East Africa Herbarium

expt.....Experiment

I_D.....Index of dispersion

IMRT.....Insect Mass Rearing Technology

K.....Index of aggregation

LSD.....Least significant difference

MN.....Mean number of eggs

MPFS.....Mbita Point Field Station

MS.....Mean square

No.....Number

Nos.....Numbers

NTM.....Netting material

Ov.....Oviposition period

OV SBT.....Oviposition substrate

Pre-Ov.....Pre-oviposition period

S.E.....Standard error

SNK.....Student-Newman Keuls

SS.....Sum of squares

TT.....Total number of eggs

CHAPTER 1

GENERAL INTRODUCTION.

The legume pod-borer, *Maruca testulalis* (Geyer) (Lepidoptera: Pyralidae) is found throughout the tropics and subtropics of Africa, Asia, Central and South America (Fig.1). It is a major pest of most common grain legumes grown in these areas; for instance it is one of the major cowpea pest complex in West Africa where the cowpea plant stays longer on the fields for the seeds to grow to full maturity and subsequently get dry. The FAO Panel during its meeting in 1975 identified *M. testulalis* as one of the most important pest of grain legumes. Its distribution and importance in relation to the various cultivated grain legumes have been documented by Singh and van Emden (1979). The importance of grain legumes especially cowpea in the African diet cannot be over-emphasised. Cowpea pods, dry seeds and leaves are all used for food in various regions where cowpea is extensively grown. In East Africa, the leaves are used as green vegetable while the dry seeds are used more widely in West Africa.

M. testulalis is a serious pest of cowpeas in various regions of Africa. In Kenya, for example, losses of up to

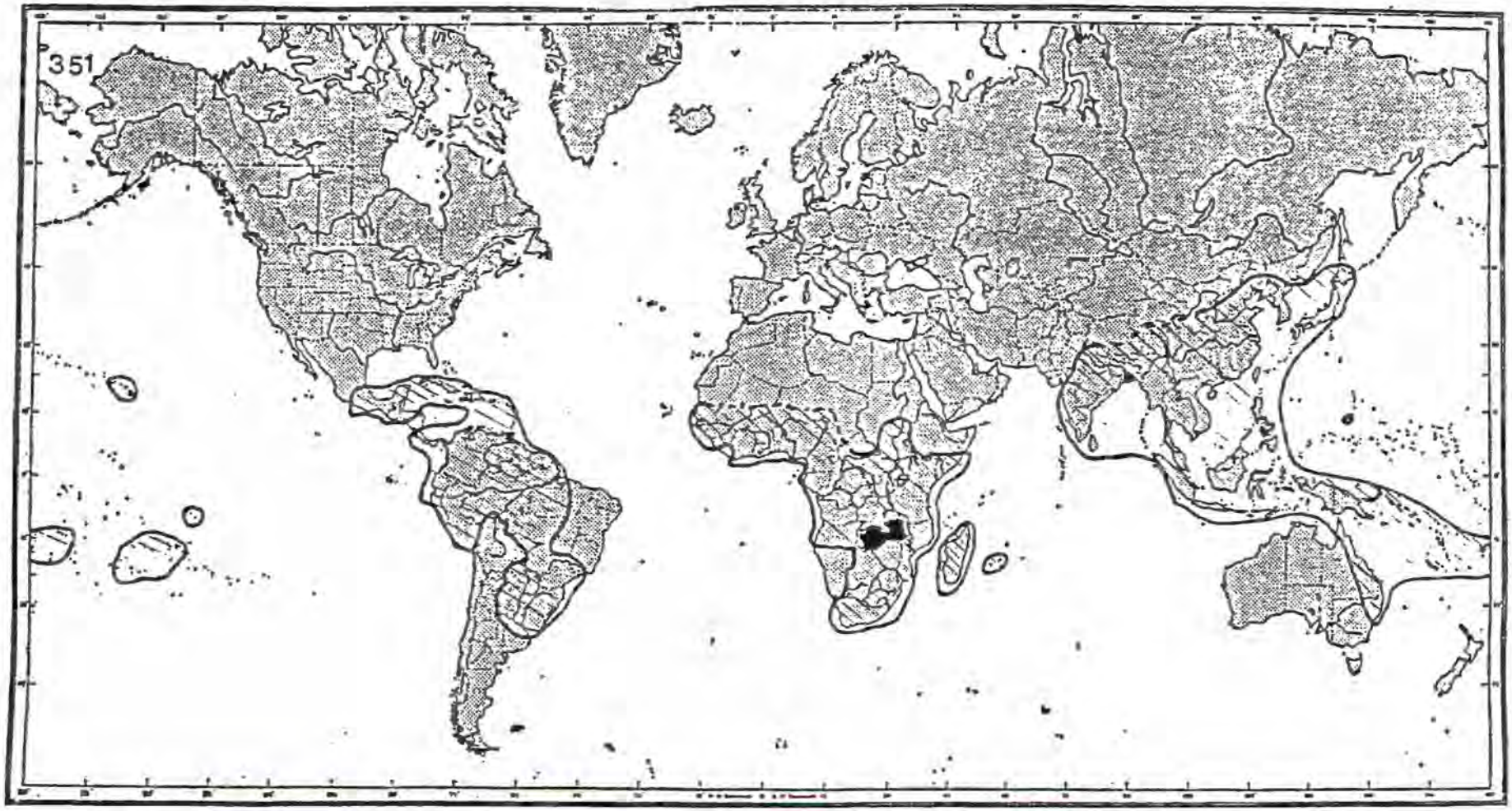

Fig. 1: Map of the world showing the distribution of *M. testulalis* (Commonwealth Agricultural Bureaux, 1975)

80% occur on indigenous cowpea varieties as a result of this pod-borer attack (Okeyo-Owuor *et al.*, 1983). In Nigeria, losses ranging from 30 to 86% on high-yielding varieties have been reported by Taylor (1978). Although Booker (1965) reported increase in cowpea yield in Nigeria of up to 1800 Kg/ha by applying chemical insecticides, the damage by this pest remained high. Moreover the use of chemical insecticides for controlling this pest is not advisable since both leaves and seeds are used for food in many areas of Africa, especially Eastern Africa. Biological control of this pest on pigeon peas in the Fiji Islands was initiated early in 1970's with practically no success (Singh and Jackai, 1988). Recent studies by Okeyo-Owuor *et al.* (1983) revealed that intercropping as a cultural control method had no effect in reducing the pest populations and damage to cowpea. Time of planting and manipulation of planting dates has not helped either (Ezueh, 1982).

The main types of behaviour which play important roles in host plant selection include (1) orientation, which determines an insect's arrival/stay on a plant or its avoidance (2) feeding and (3) oviposition. Selecting oviposition sites is the sole responsibility of gravid females. A thorough understanding of this moth's behaviour may therefore be quite useful in initiating better and sustainable control strategies. But, the

available information on *M. testulalis* behavioural patterns is barely sufficient to facilitate development of realistic pest management strategies for this legume pod borer (Singh and Jackai, 1988). It is therefore essential to study the field biology of *M. testulalis*, especially its mating and oviposition behaviours. In this work emphasis was placed on oviposition behaviour. The chain of behavioural events leading to oviposition parallels those used in food location (Matthews and Matthews, 1978). Because oviposition mistakes can severely reduce larval survival, the sequence of stimulus-response events leading to oviposition are adapted to maximize larval survival. Plant factors influencing gravid moth behaviour include both physical (temperature, humidity and photoperiod) and chemical stimuli, especially host-plant semiochemicals. Lewis and Nordlund (1985) reviewed the effects of behaviour-modifying chemicals (semiochemicals) produced by plants (synomones) and by host or prey arthropods (kairomones). The potential of semiochemicals in increasing the effectiveness of biological control measures against agricultural pests was discussed by Lewis and Nordlund (1985) and shown to be feasible in some pests.

A range of plant-derived factors are known to inhibit or stimulate oviposition responses (Matthews and Matthews, 1978). An example is seen where propylcysteine

sulphoxide, a precursor of volatile substances in the host plant of the leek moth, *Acrolepiopsis assectella*, was reported to have reduced oviposition, while a methanolic solution of crushed leek stimulated egg-laying as much as a piece of the leek plant itself (Thibout *et al.*,1982). Females of the Pyralidae, *Dioryctria amatella*, a pest of pines, have been reported to oviposit more frequently on materials with high monoterpene contents instead of selecting those plant materials prefered by its larvae for feeding. A synthetic mixture of monoterpenes similar in composition to those present in pine elicited mating and oviposition in those females (Fatzinger and Merkel, 1985). For *M. testulalis* such information is lacking and this calls for concerted behavioural chemical investigation to generate data that can be employed in the integrated control strategies of this pest.

An efficient mass-rearing technique which can ensure production of large numbers of *M. testulalis* is a prerequisite for the effective screening of host plant resistance. Although an artificial diet has been developed for the mass-rearing of *M. testulalis* at the Insect Mass Rearing Unit of the International Centre of Insect Physiology and Ecology (ICIPE) (Ochieng and Bungu, 1983), there is still the problem of a satisfactory artificial oviposition substrate. Mass-rearing *M. testulalis* still relies on eggs deposited on young cowpea

plants because the moths prefer ovipositing on cowpea leaves. This is a limiting factor because fresh cowpea plants are required all the time. Development of an efficient artificial oviposition substrate can go a long way in reducing the cost of rearing *M. testulalis*. In order to achieve this objective, proper oviposition behaviour studies are essential. The present study was therefore aimed at getting detailed information on the oviposition behaviour of this moth. The objectives were as follows:

1) Examine the oviposition behaviour of gravid moths on host and non-host plants.
   (a) Describe the oviposition process of gravid moths, and the pattern of egg placement.
   (b) Identify the main sensory structures involved in identification and selection of oviposition sites.
   (c) Describe the ovipositor apparatus in relation to the oviposition substrates.
2) Identify factors stimulating or deterring oviposition:
   (a) Physical stimuli/deterrents
   (b) Chemical stimuli/deterrents types by bioassaying: host and non-host plant extracts.

3) Testing of potential artificial substrate for oviposition.

CHAPTER 2

LITERATURE REVIEW

2.1 OVIPOSITION BEHAVIOUR IN INSECTS

Oviposition behaviour consists of a sequence of stimulus-response interactions resulting in host plant selection and pinpointing of oviposition sites which can ensure eggs and subsequent larval survival. Some insect species are very particular as to where they lay their eggs while others are not. For example, *Chilo partellus* females are not specific with respect to where they deposit their eggs (Kumar and Saxena, 1985a). The number of eggs produced and where they are deposited also plays an important role in ensuring that there are sufficient numbers of offsprings to establish and sustain the insect species (Saxena, 1969). Behavioural events culminating in oviposition are guided by several sensory cues (Miller and Strickler, 1984), such as plant semiochemicals which consists of volatile compounds (Yamamoto and Fraenkel, 1960; Salama *et al.*,1984); leaf surface contact chemical stimuli (Gupta and Thorsteinson, 1960; Stadler, 1978; Renwick and Radke, 1983; Jackson *et al.*, 1984; Stadler and Schoni, 1990) and surface texture of the oviposition

substrate (Hagley *et al.*,1980). For example host-plant factors influencing the oviposition behaviour of *Etiella zinckenella* Treitshke have been studied by Hattori (1986, 1988). Among the factors affecting this moth's oviposition are the following: odour, water vapour, mechanical stimuli and contact chemostimuli. Furthermore this moth displays oviposition behavioural patterns common to many moths. It was observed that gravid females oviposited several eggs on a plant at each arrival. A female that alighted on a host plant bent her abdomen downwards immediately after touching its surface with her antennae, and began to walk forward while dragging her ovipositor tip and drumming the surface intermittently with her antennae. On detecting a suitable area, she stopped walking and raised the anterior portion of her body. She then occasionally searched the surface of the area intensively with the ovipositor tip alone and then deposited an egg. The moth often repeated this sequence several times, laying an egg each time. These oviposition bouts were repeated in the early part of the dark period, interspersed with flying and resting activities (Hattori, 1986). In order to appreciate the unique oviposition behaviour patterns displayed by various moths, it is essential to review the roles played by the following aspects: environmental factors, plant characteristics and sensory organs.

2.2 FACTORS AFFECTING OVIPOSITION BEHAVIOUR

2.2.1 Environmental conditions

As stated by Banerjee and Decker (1966), the activity of many lepidopterous and other insects is governed primarily by light while humidity and temperature are among the other physical environmental factors known to influence insect activity. Light has been found to be one of the basic physical factor regulating insect activity (Edwards, 1964; Harker, 1961). Insects respond to daylight/darkness and synchronize activities such as mating, oviposition and feeding accordingly. Observations carried out in the laboratory by Banerjee and Decker (1966) showed that egg laying in *Crambus trisectus* occurred at 24-hour intervals and females failed to respond properly when conditions were changed from light to dark in less than 16 hours . It was observed that if longer intervals were maintained, females could be stimulated to lay eggs at any hour of the day by changing the conditions from light to darkness. Continous light at the level of the insects reduced oviposition in the Indian-meal moth, *Plodia interpunctella* (Hubner) by 50-75% of that normally obtained in the dark (Lum and Flaherty, 1970). The period of daylight seems to be of considerable significance in determining the time oviposition commences. Lights-off in the laboratory which can be

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compared with "dusk" serves as a stage, setting a cue for egg release by gravid female moths. The available data suggest that the activity rhythm is related to the effects of a change from light to dark conditions on the secretory cycle of the subeosophageal gland neurosecretory cells (Harker, 1960).

2.3 PLANT CHARACTERISTICS

2.3.1 Physical Properties-tactile/visual stimuli

Plant physical properties known to influence host plant selection behaviour of insects include tactile and colour stimuli. Ampofo (1985) and Kumar and Saxena (1985b) observed that trichomes on the leaf surfaces can influence the selection of oviposition sites by *C. partellus* moths. According to Gupta and Thorsteinson (1960), the diamondback moth, *Plutella maculipennis* laid much more eggs on a pebbled, grooved or pitted polyethylene surface than on a smooth substrate of the same material even when there was no olfactory stimulus.

Hattori and Sato (1983) examined the factors involved in the oviposition response of the limabean pod borer, *E. zinckenella* using soybean plants as the substrate. Gravid females were observed to choose ovipositing among hairs on the plant surface in a pubescent variety, while they

oviposited preferentially under the sepal and stipels in a glabrous variety. Their results suggested that the physical texture and moisture of the substrate strongly evoke oviposition in *E. zinckenella*. Sosa (1988) observed that pubescence in sugarcane plant affected oviposition and mobility in the sugarcane borer, *Diatraea saccharalis*. Other plant physical factors which affect or influence choice of oviposition site include leaf shape (Rausher, 1978; Stanton, 1982), fruit size (Katsayonnos and Pittara, 1983) and colour (Sternlicht, 1974; Saxena and Goyal, 1978; Scherer and Kolb, 1987). The morphology of the petals of *Melandrium album* were found to affect oviposition in *Hadena bicruris* (Brantjes, 1976). By inserting their proboscis into the tubular corolla, these moths were able to identify the sex of the unisexual flower. These moths usually oviposit in the female flowers. *Prays Citri* females preferred ovipositing on blue, yellow, white, black and red surfaces in that order (Sternlicht, 1974), while green or yellowish-green colour attracted *Papilio demoleus* females most (Saxena and Goyal, 1978). There is now substantial evidence showing that the resistance of cretain plants to insect attack is dependant on leaf surface patterns (pubescence) (Beck, 1965; Lyman and Cardona, 1982). For instance, Callahan (1957) reported that the ovipositing *Heliothis* female grasps protruding ribs made by the leaf veins; but when this are far apart as in the case of the glabrous leaf, egg laying

is prevented (Southwood, 1986)

2.3.2 Semiochemicals

Chemical characteristics influencing oviposition behaviour in insects may be close- or long-range. Hattori (1988) reported that the oviposition behavioural sequence of *E. zinckenella* depends on plant odour, water vapour and mechanical stimuli. Water vapour causes an alighting response by the female moths in the vicinity of the plant, while plant odour and water vapour together induced "abdomen-bending walking" (scanning with ovipositor) on the plant and finally, mechanical stimuli induced egg deposition. Hamilton *et al.* (1978) identified a hydroxy-B-diketone as the chemical responsible for the attraction of *Oscinella frit* to oats for oviposition. The experiments demonstrated an olfactory response which could occur at some distance from the leaf surface. Kumar and Saxena (1985b) and Kumar (1988) examined the ovipositional responses of *C. partellus* on certain susceptible and resistant maize genotypes. It was found that certain characters of the plants eliciting ovipositional responses were detectable prior to moths' arrival on the plants (distance perceivable) while others were detectable after arrival on the plant (contact perceivable). Distance perceivable characters were not found to be responsible for the differences in the oviposition responses on the

two host-plant cultivars tested, however contact-perceivable characters were responsible for the observed differences in the oviposition responses.

The importance of direct observation in behavioural studies was exemplified by the work of Katsoyannos and Pittara (1983) in their studies on the selection of oviposition site by the olive fruit fly, *Dacus oleae*. When this fly was presented with artificial and natural oviposition substrates, egg counts alone indicated strong preference for artificial domes over the olives. However, direct observation revealed that the females began laying eggs on the dome only after the olives have received a number of eggs and had been marked by an oviposition-deterring factor (ODF). The ODF acted only on the olives but not on the artificial domes. This was because in the case of the artificial domes there was no release of ODF so oviposition was not deterred. The ODF was probably obtained from the olives after one or two oviposition punctures (Cirio, 1971).

Schoni *et al.*, (1987) studied host and non-host plant chemicals influencing the oviposition behaviour of several herbivorous insects. The oviposition behaviour and sensory physiology of adult insect species having different host specificities were compared using the behaviourally active compounds from cabbages. The

influence of compounds extracted from cabbage leaves on the oviposition behaviour of two "specialists" cabbage pests, *Delia radicum* and *Pieris rapae*; a "generalist" cabbage pest, *Trichoplusia ni* and the carrot fly *Psila rosae* were examined. It was revealed that the decision to accept or reject a plant as a suitable host was not uniquely based on some few key stimuli, e.g. the glucosinolates, but rather on a large variety of stimulatory and inhibitory plant chemicals acting together. In addition, it was reported that the effective chemicals appeared to be specific for each species (Schoni *et al.*, 1987). In the presence of such chemicals it was observed that leaves of non-host plants perfused with sinigrin induced oviposition in *Delia brassicae* (Woodhead and Chapman, 1986). *Pieris brassicae* which normally oviposits on plants of family *Cruciferae*, was stimulated to oviposit on green paper treated with 0.2% sinigrin (David and Gardiner, 1962). Oviposition by *Psila rosae* on filter paper "leaves" was induced by a number of chemicals obtained from the wax of carrot leaves. Those chemicals have been identified as propenylbenzenes, furanocoumarins and polyacetylenes and are located in the epicuticular wax. Such chemicals provide the final element in host recognition before oviposition by a number of phytophagous insects (Stadler and Buser, 1982; Stadler, 1986; Woodhead and Chapman, 1986).

2.4 SENSORY ORGANS

Each insect has a physiologically characteristic sensory receptive system that is never completely identical with that of any other species (van der Pers, 1982). Sensory receptors concerned with mating and oviposition behaviours especially olfactory receptors functionally adapted to respond to airborne volatiles are located in most insects on the antennae (Visser, 1986). Relatively few of these sensilla are found on other appendages (Schoonhoven, 1973; Schoonhoven and Dethier, 1966). Zacharuk (1985) reported that among chemosensory organs, the antennae have the greatest concentration of multiporous pitted and multiporous grooved sensilla. The antennae are thus the primary olfactory organs responsible for detecting long-and short-range stimuli from mates and host plants.

Calvert and Hanson (1983) found that the antennae and foretarsi of the patch butterfly, *Chlosyne lacinia* were involved in host identification and oviposition and that behavioural observations strongly implied that the sensory structures on these organs participated in the selection of the oviposition site. The biological significance of the drumming behaviour (using the antennae) and the tapping movements of the foretarsi in moths or butterflies enhance the perception of non-volatile chemicals present

in the leaf or oviposition substrate (Ma and Schoonhoven, 1973). Innervation of the lepidopteran tarsal sensilla shows that they have contact chemoreceptors. Waladde *et al.* (1985) reported that the first tarsomere on the foretarsi of *C. partellus* has three pairs of sensilla; two of them having similar ultrastructural details innervated by gustatory cells which respond similarly to maize leaf exudate but differing in their sensitivity to sucrose. In the cabbage white butterfly, *Pieris brassicae*, the tarsal contact chemoreceptors have been implicated in the selection of oviposition sites (Ma and Schoonhoven, 1973).

It has been suggested that the ovipositors of some moths and butterflies monitor the surface texture of the oviposition substrate (Thorsteinson, 1960; Gupta and Thorsteinson, 1960; Yamaoka *et al.*, 1971; Calvert and Hanson, 1983; Klijnstra, 1982, 1985). Among the large number of typical mechanoreceptor hairs on the tip of the ovipositor valves of *C. partellus*, two pairs of stout, blunt-tipped hairs were discovered by Chadha and Roome (1980). These sensilla are contact chemoreceptors and they are positioned in such a way that they come in contact with the ovipostion substrate during egg placement. Similar hairs have been found on the ovipositor of *Spodoptera littoralis* (Chadha and Roome, 1980). It was suggested that the hairs may prevent oviposition on surfaces chemically harmful to eggs.

Waladde *et al.* (1985) carried out functional morphological studies and electrophysiological tests on larval and adult stages of *C. partellus* with materials obtained from susceptible and resistant maize cultivars. It was shown that taste sensilla on the ovipositor were more sensitive to NaCl than sucrose and that they responded to some unknown components in the maize leaf exudate. When leaf exudate was forming on the upper surface of the maize leaves, the moths chose oviposition sites on the under-surface of the leaves (Waladde *et al.*, 1985). It is possible that the chemical components present in the leaf exudate may be one of the factors preventing oviposition on the upper surface of the leaves, since the ovipositor sensilla responded positively to chemical stimuli in the leaf exudate (Waladde *et al.*,1990).

2.5 BIOLOGY AND ECOLOGY OF M. TESTULALIS

2.5.1 General behaviour

Taylor (1978) and Jackai (1981a) reported that *M. testulalis* moths deposit their eggs on the flowers and flower buds, while studies at the Mbita Point Field Station (MPFS) indicate that oviposition occurred on all the aerial parts of the cowpea plant, with the leaves being the most preferred sites (Okeyo-Owuor and Ochieng, 1981). Variations in oviposition behaviour may be due to

differences in *M. testulalis* biotypes in various regions of Africa (Okech, 1986) or in experimental procedures used by different workers. However Jackai and Singh (1991) maintained that oviposition on host leaves may occur only in the absence of reproductive structures i.e flowers and flower buds. Neonate larvae as well as latter larval stages feed on tender plant stems, terminal shoots and peduncles during vegetative stage of plant growth. Older larvae (4th instar upwards) are quite mobile; they feed particularly on flowers and newly formed pods and cause severe damage throughout the reproductive stage of the crop (Jerath, 1968; Akingbohungbe, 1982; Okeyo-Owuor *et al.*, 1983; Singh and Taylor, 1978; Jackai, 1981b; MacFoy *et al.*, 1983). According to Jackai (1981b) larval infestation is highest in the flowers, followed by flower buds, terminal shoots and pods. Pods located within the leaf canopy and those touching each other as well as other plant parts are heavily infested and sustain severe damage (Usua and Singh, 1979).

Okeyo-Owuor and Ochieng, (1981) reported that *M. testulalis* moth activities at Mbita began at 1800 hours and ended 0500 to 0630 hours. They also observed that both natural and artificially induced darkness would initiate adult activity. Okeyo-Owuor (1988) and Okech (1986) also mentioned that *M. testulalis* started activities as darkness approached at Mbita. Akinfenwa's

(1975) data showed that moth activity was between 1840 and 0450 hours with peak flight activity occurring between 2000 and 2100 hours. IITA research highlights (1981-1984) puts adult activity at 1900 hours and peaks between 2200 and 0200 hours. However none of the above workers gave any definite data about the commencement of oviposition activities by this moth.

2.5.2 HOST PLANTS

Cowpea, *Vigna unguiculata* is the most important pulse crop in tropical Africa, providing protein, calorie energy and vitamins in human diet (Usua and Singh, 1979). Behavioural bioassays with volatiles and chloroform/n-hexane extracts of cowpea leaves, flowers and pods of the susceptible VITA 1 variety showed that these were more attractive to *M. testulalis* larvae than those from the resistant cultivar Tvu 946 variety (Okech, 1986). There are reports that *M. testulalis* gravid females oviposit more on VITA 1, the susceptible cultivar, when presented in a choice situation with either Tvu 946 or VITA 5, the resistant varieties (Okech, 1986). Studies on the chemical basis of host plant selection of *M. testulalis* have been carried out using larvae. Although feeding bioassays testing larval responses to extracts from resistant (Tvu 946) and susceptible (VITA 1) cowpea cultivars have been carried out (Otieno *et al.*, 1985;

Okech, 1986 and Mugoya, 1991), there is limited information on the physical and chemical factors facilitating host plant recognition by the ovipositing moth.

Taylor (1978) listed host plants of *M. testulalis* but did not distinguish which of them serve as maintenance hosts, normal food sources or trap crops which can attract moths for oviposition but cannot support larval development.

However, Jackai and Singh (1983) in their studies on the suitability of various leguminous plant species for oviposition reported that *Vigna unguiculata* (cowpea) was the most suitable host plant while *Crotalaria juncea* L. attracted heavy oviposition but could not support larval development beyond 3rd instar stage. Okeyo-owuor's (1988) survey at Mbita listed *Sesbania sesban*, *Vigna vexillata* and *Crotalaria* sp. as wild host plants of this pest. In Nigeria, several wild leguminous shrubs and trees constitute the main foci of *M. testulalis* during dry season months when the principal host plant is not cultivated (Usua, 1975; Taylor, 1978; Singh, 1978). Singh (1980) reported that population of this moth were lower in field-plots of cowpea with *Crotalaria juncea* planted along the borders. *C. juncea* probably provides adequate physical and chemical stimuli for oviposition by *M. testulalis*, although it is reported not to support larval

feeding and survival (Jackai & Singh, 1981, 1983). Similarly, Kogan (1978) had reported that the moth, *Autographa precationis* oviposited on a plant which could not sustain its larvae. Akinfenwa's (1975) study on host range of *M. testulalis* in Northern Nigeria, implicated cowpea as the most susceptible amongst a number of cultivated and wild legumes. Out of nine species of wild plants belonging to family Papilionaceae, *Crotalaria lachnosema* which flowers all year round was the most important alternative host found in Northern Nigeria.

CHAPTER 3

GENERAL MATERIALS AND METHODS

3.1 LABORATORY CONDITIONS

The experiments conducted in the laboratory were carried out in a room with controlled temperature and humidity conditions. The light regime of 12L:12D (Fig.2) in the room was provided by a 65/80 watt fluorescent tube which automatically switched off at 9000 hours and turned on at 2100 hours by means of a timer (Orbis alpha). Temperature was maintained between 24-30 ^{0}C by a Philips fan heater (Twin Turbo 3); and relative humidity was maintained at 70-85% by using a 505 Defensor humidifier with its automatic control set at 70% r.h.

3.2 INSECTS

The moths, *Maruca testulalis* (Geyer) were obtained from the Insect Mass Rearing Technology (IMRT) unit at ICIPE's Mbita Point Field Station (MPFS). They were supplied to the laboratory in the pupal stage contained in glass vials (7.5 x 2.5cm) (Plate 1). These vials were transferred into emergence cages made of mosquito netting

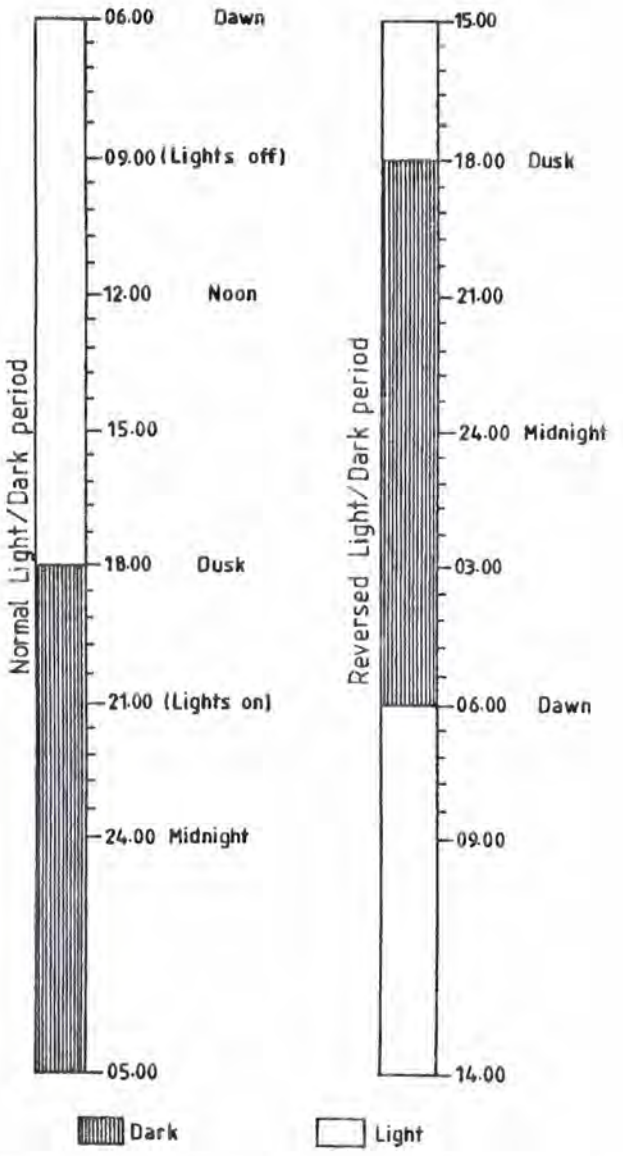


Fig.2. Chart showing light - dark periods in the control room

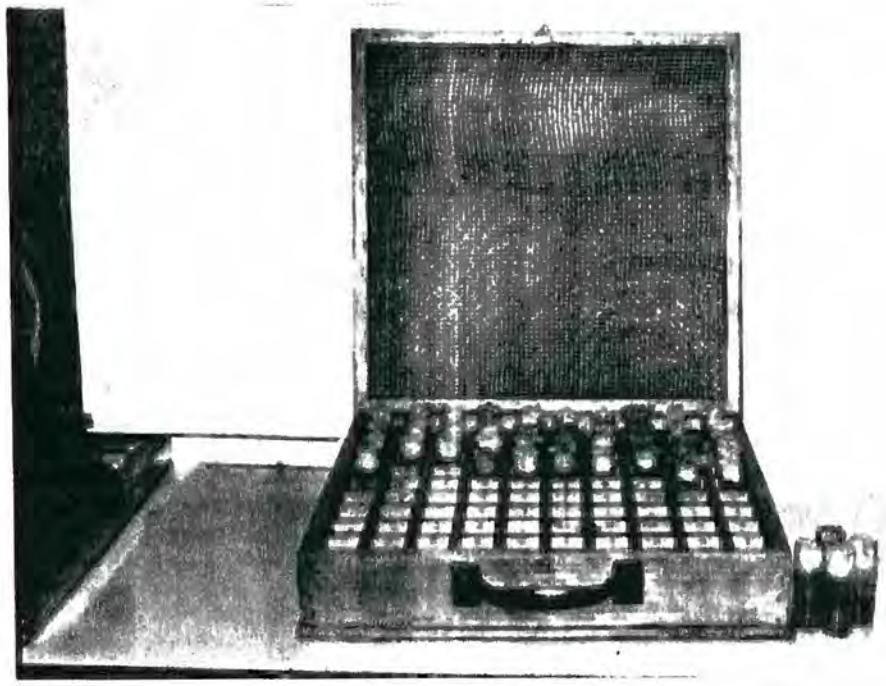


Plate 1: Wooden crate containing cotton-plugged glass vials bearing M. testulalis pupae in an artificial diet

material (Plate 2), and placed in a room with controlled temperature and humidity conditions. On the first day of emergence, the moths were sexed and kept in separate compartments where they were fed on 10% sucrose solution (Plate 3). Those moths served as the stock animals for the experimental work. When already mated females were required for any experiment, batches of fifteen males and fifteen females were paired on the second day after emergence. Mated moths were ready to oviposit on the second day after mating i.e forty-eight hours after mating.

3.3 PLANTS

The seeds of *Vigna unguiculata* L. Walp. (cowpea cultivar, VITA 1), the host plant of *M. testulalis*, were obtained from the ICIPE's Mbita Research Station. A non-host plant, *Gossypium barbadense* L. (cotton) was obtained from a student's farm at Mbita while the trap crop, *Crotalaria juncea* L. (sunhemp) seeds were provided by the IITA and also obtained from Columbia. The three plants were grown in experimental plots at the MPFS and in the greenhouse at Chiromo, Nairobi. At Chiromo and in the laboratory at Duduville, the test plants were grown in flower pots and cups measuring 14cm. and 7cm. respectively. Plant specimens were sent to the National Museum of Kenya, East Africa Herbarium for proper

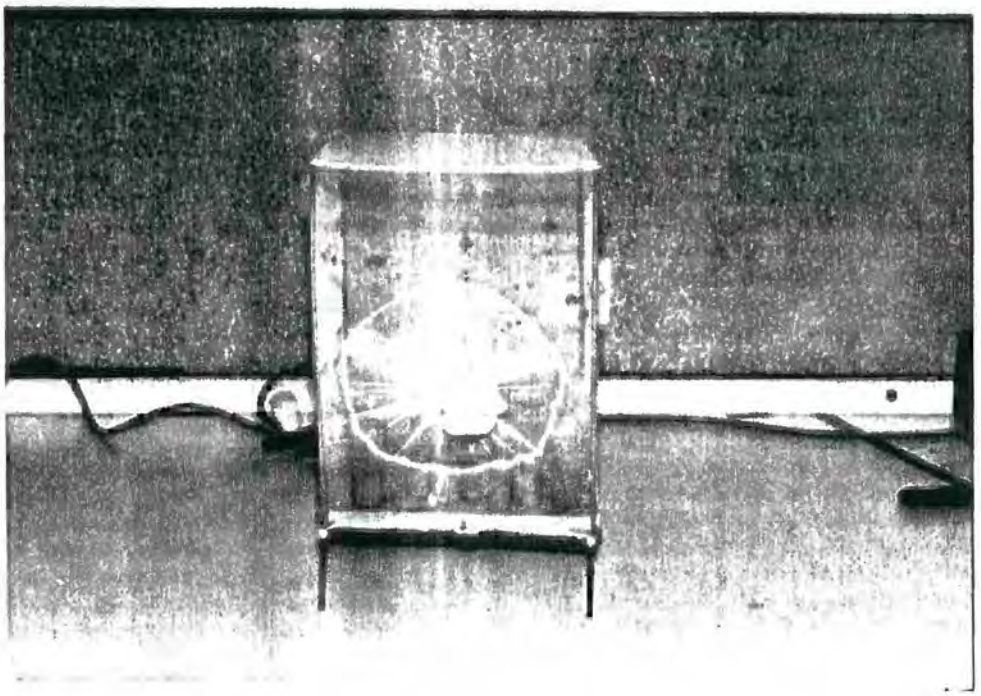


Plate 2: Emergence cage containing glass vials with emerged moths perching on the mosquito netting

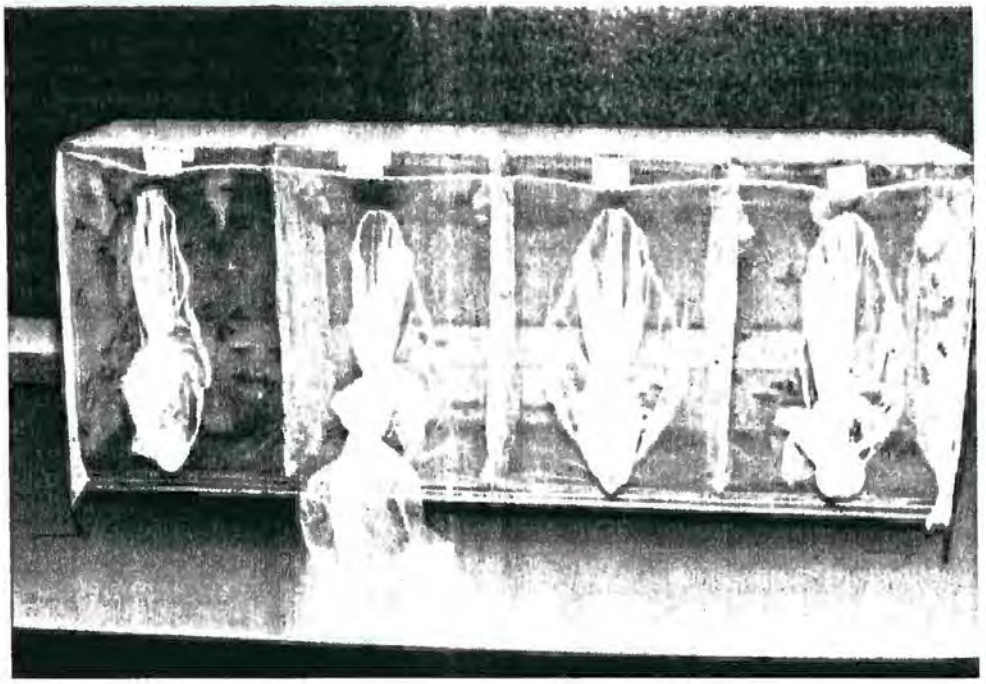


Plate 3: Four-compartment cage used for keeping sexed moths

identification (Appendix 2). All plants used in this work were those grown from seeds of the identified plants and they were used at two/three weeks of age.

3.4 EXPERIMENTAL CAGES

Cages used in this study depended on the experiment and are described under the appropriate sections. They varied from cages made out of mosquito netting to those fabricated using perspex and their sizes depended on the experiment as mentioned above.

CHAPTER 4

PATTERNS OF M. TESTULALIS OVIPOSITION BEHAVIOUR

4.1 INTRODUCTION

Observations on behavioural manoeuvres can be used to investigate factors related to genetics of oviposition behaviour, chemistry of host choice (Thompson and Pellmyr, 1991) and the sensory inputs associated with this behaviour. Before any of the three areas are investigated, it is essential to establish some basic information which can serve as starting points for such investigations. Since there is not much information on the patterns of *M. testulalis* oviposition behaviour, the objectives of the work reported here were relatively broad and are as follows:

i) Examine and describe oviposition response of *M. testulalis* on the host (cowpea, *Vigna unguiculata* (VITA 1)), wild host (sunhemp, *Crotalaria juncea*) and non-host (cotton, *Gossypium barbadense*) plants in a choice situation. This was done under laboratory controlled conditions which also provided an opportunity to assess the suitability of those conditions for subsequent experiments. Cotton, the non-host plant, was chosen for

comparison since it is a non-legume, grows well in areas where cowpeas and beans are grown and it is often intercropped with cowpea. Furthermore its phenological and physiognomic development features compare well with those for the host plants of *M. testulalis* (Mugoya, 1991).

ii) Undertake field observation and compare them with laboratory results. With this information, basic data on the conditions required to carry out further investigations were established.

iii) Ascertain the spatial distribution pattern of eggs on natural and artificial oviposition substrates.

4.2 MATERIALS AND METHODS

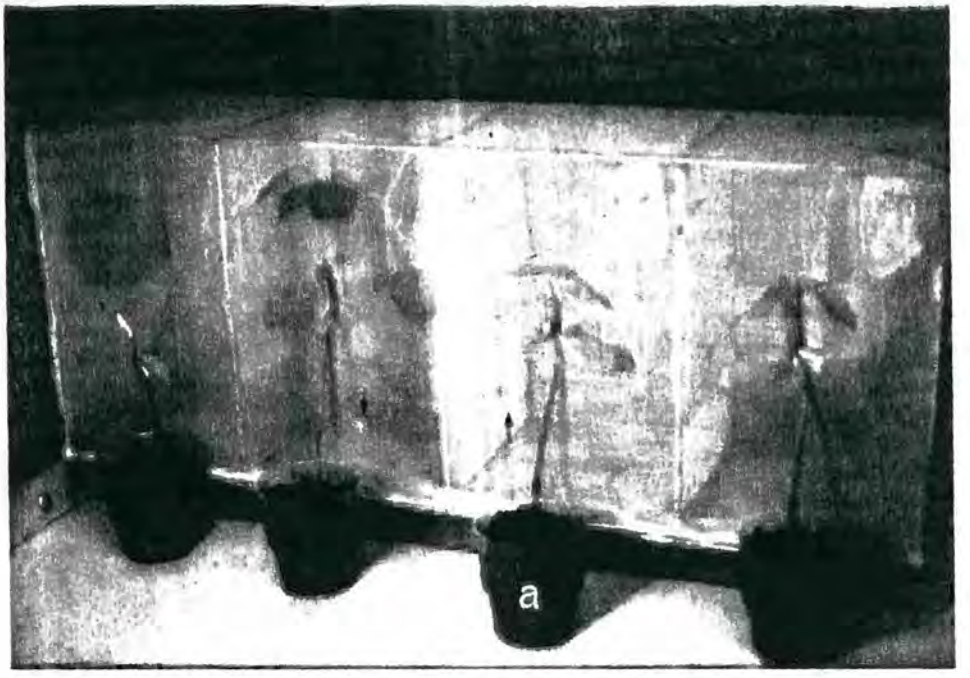
4.2.1 Oviposition response on host, non-host and wild host plants (choice test)

Seven pairs of 2-day old moths (7 females:7 males) were released in a 23 x 23 x 35 cm mosquito-netting cage. In the choice test the moths were presented with the three test plants (cowpea, cotton and sunhemp) planted in plastic pots and they were fed on 10% sucrose solution applied on cotton-wool. From the second day of setting up the experiment, when the moths were 4-days old, and every successive two days, the number of eggs laid on each of

the plants was recorded. The experiment was allowed to run for 18 days. The egg counts were transformed using log transformation and two-way ANOVA was carried out on the transformed data to detect significant differences between days and plants.

4.2.2 Effect of photoperiodism on oviposition behaviour of *M. testulalis*

Several pairs of moths were selected on the first day of emergence and kept in the same cage. On the third day mating pairs in that lot were picked from the rest and placed in separate containers used for this experiment. Seven mated females were used per cage. The mated female moths were placed in 15 x 15 x 30 cm net cages and provided with 10% sucrose solution applied on cotton-wool. A set of leaves on a potted 2-weeks old cowpea plant was introduced into the cage in such a way that only the leaves were inside the cage (Plate 4). The cowpea plant was replaced every two hours starting at 1000 up to 0200 hours (normal time) i.e. two hours before lights-off and two hours after lights-on. The plants placed in at 0200 hours were left in the cages until the next day, when they were again replaced at 1000 hours The experiment was run for 5 days and had four replicates. The number of eggs deposited on the leaves were recorded and analysed.



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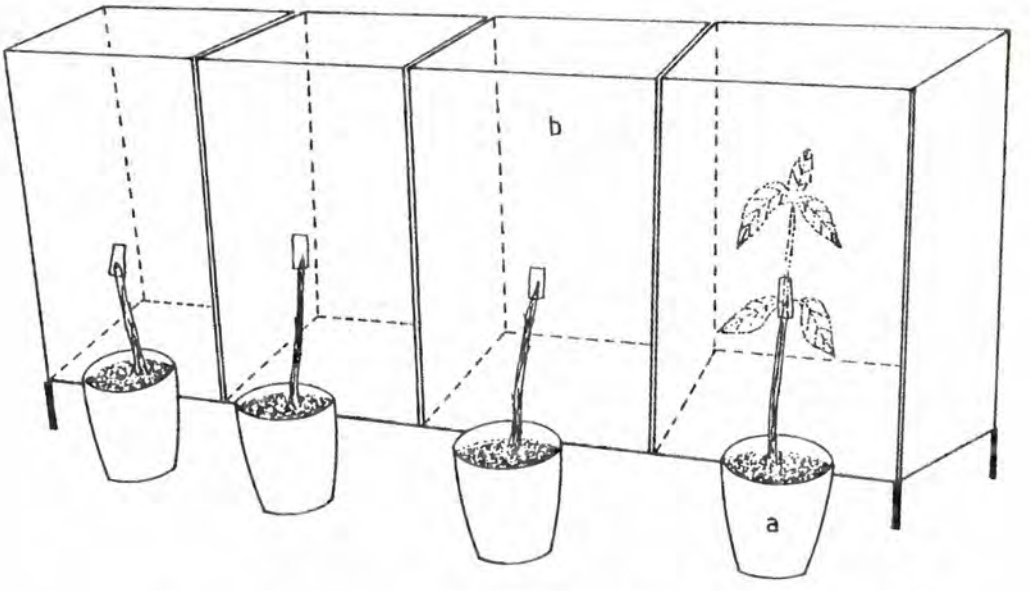

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Plate 4: (i) Potted cowpea plants with leaves introduced into 4-compartment cage. (ii) Schematic diagram of (i) (a, potted cowpea with leaves introduced into cage; b, 4-compartment net cage, arrow - moths)

4.2.3 Sequence of events leading to egg deposition

Laboratory observations

A number of two-day old male and female moths were paired in 15 x 15 x 30 cm cages. They were provided with 10% sucrose. At intervals of 30 minutes from lights-off up to eight hours in scotophase, mating pairs (Plate 5) were picked using vials and kept in holding glass vials (7.5 x 2.5 cm) with perforated plastic lids. Each vial contained one mating pair. On the following day, i.e one day after mating, a mated female was transferred from the holding vial into an observation perspex cage, 6 x 3 x 15 cm containing a cowpea leaf still attached to the potted plant and held in place with selotape (Plate 6). The above experimental set-up was put together an hour before lights-off. The moth was observed from lights-off up to six hours after lights-off. Activities culminating in egg deposition on the oviposition substrates i.e leaf/wall of cage were verbally recorded on a Racal Store 4 DS instrumentation tape recorder. These observations were repeated four times under the same conditions. The information on the tape was later transcribed and analysed. This same set-up and procedure was used to observe the moth's responses on non-host plant (cotton)

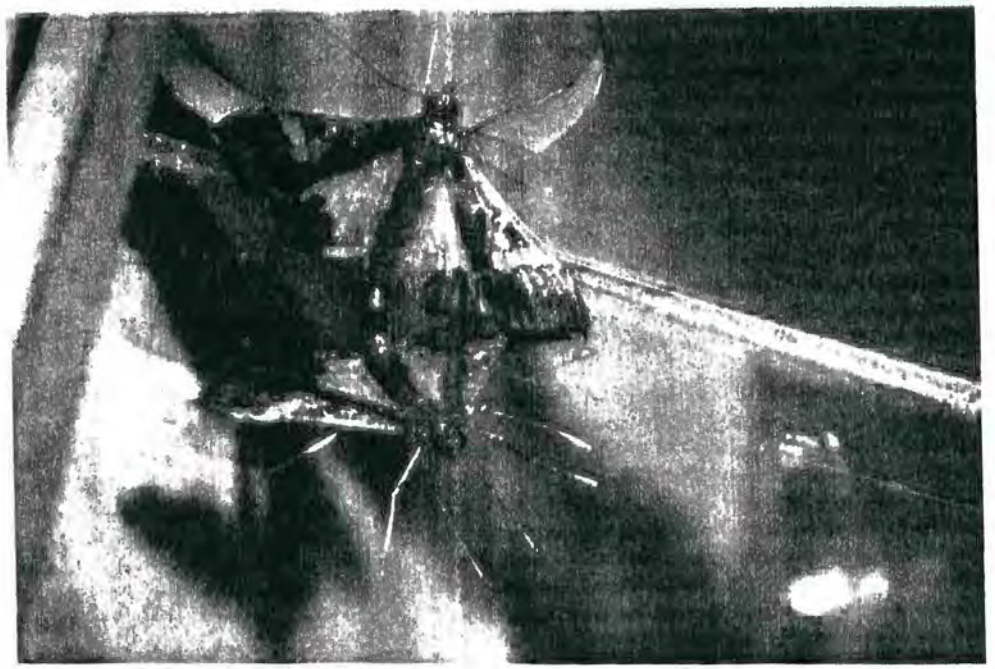
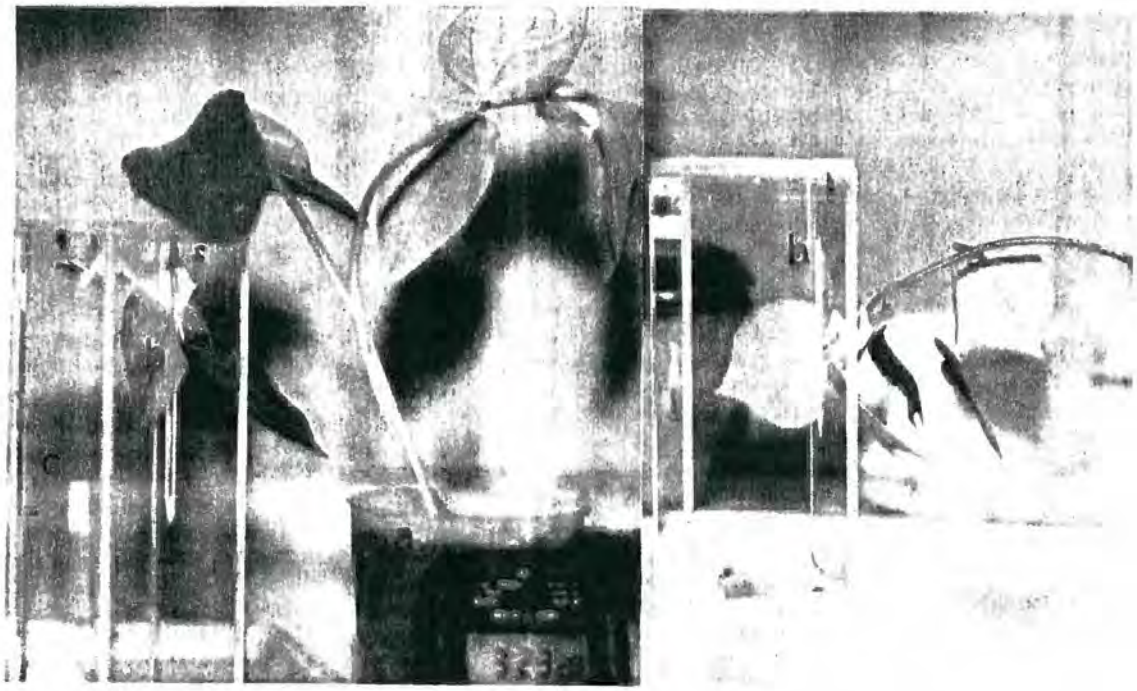


Plate 5: A mating pair of M. testulalis moths



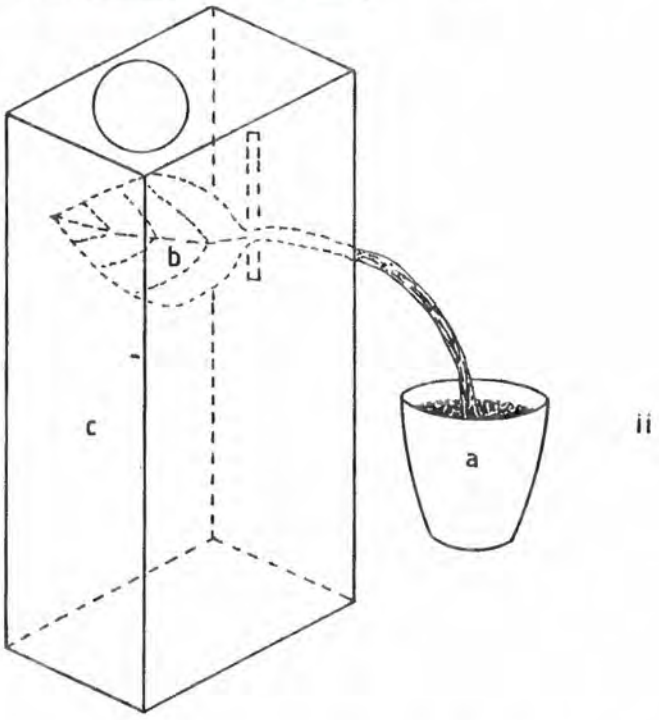


Plate 6: (i) Set - up for observing oviposition behaviour of *M. testulalis*.
(ii) A schematic diagram of (i) (a, potted cowpea plant, b, leaf introduced into cage; c, perspex observation cage, arrow - moth)

Field observations

Wire mesh cages measuring 100 x 100 x 160 cm were placed in the field at Mbita Point Field Station. Two mated 3-day old female moths were introduced into the cages containing two potted host plants (cowpea). A thermohygrograph was provided to monitor the prevailing temperature and humidity during the observations (Plate 7). The temperature during field studies was between 21 and 33 ^{0}C, while humidity was 61-94% rh. The moths were introduced a day after mating. They were observed from 1800 hours up to 0300 hours for three days. The experiment was repeated three times.

In a second group of experiments, three field cages of the same dimension as that described above were set with different combination of host and non-host plants. Cage 1 contained two pots of cotton plants, cage 2 had one pot of cowpea and one pot of cotton plants while cage 3 had two pots of cowpea plants. All observations were done using spot lights with red filters which did not seem to disturb moth activities. All moth movements during the observation period lasting six to eight hours (1800 to 0020 hours) were recorded on a radio cassette tape recorder. Areas where moths showed behaviours associated with egg deposition were marked. The leaves on all plants used had labelled tags to facilitate easy identification during night observations (plate 8). The following

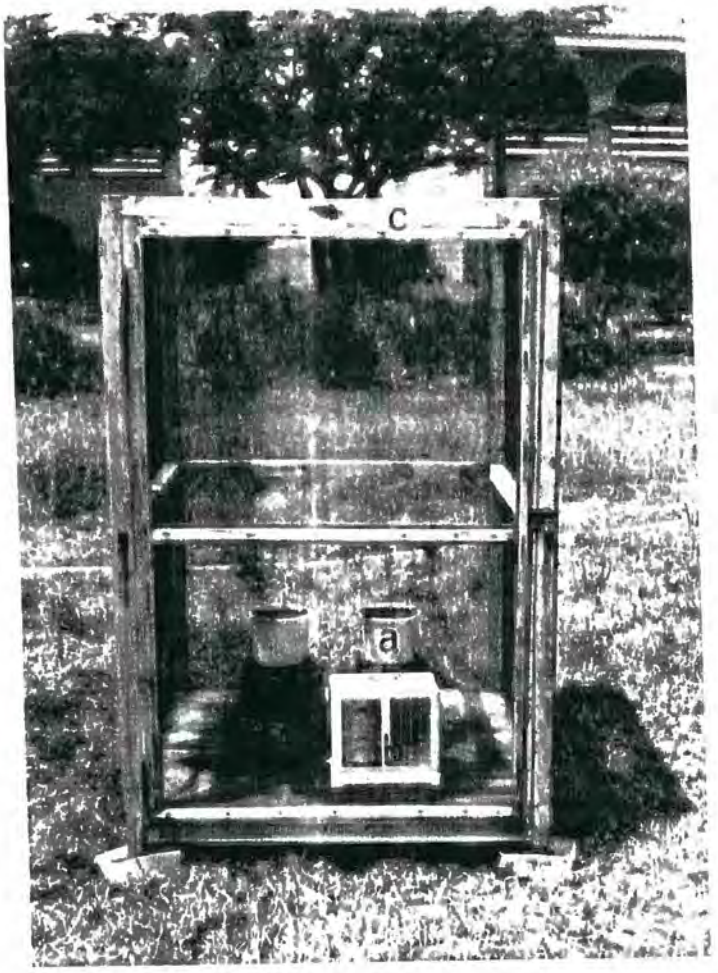


Plate 7: Field cage (a, potted plants; b, thermohygrograph; c, wire-mesh cage)

Plate 8: Potted cowpea plant with labelled tags (arrows)

morning, after the overnight observations, the cages and plants/leaves were examined for eggs which were then counted and recorded. The experiment ran for five consecutive days.

4.2.4 Pattern of egg distribution on experimental cages and leaves

A five-day old mated female moth was introduced into a 20 x 6 x 10 cm perspex cage containing a two weeks old cowpea plant. After 24 hours the eggs deposited on the cage and leaves were counted and recorded according to their location in the units. The cages were divided into cells of 3 x 2 cm units while the leaves were divided into two sides per leaf surface and a frequency table (Appendixes 4-7) was prepared. Using this frequency table, the mean number of eggs per cell as well as the variance were obtained. To determine whether the spatial distribution of eggs on the cages or leaves is random or not, the dispersion index (I_D) was obtained as

$$I_D = S^2(N-1)/x$$

where S^2, N and x are respectively the variance, number of cells and mean (Southwood, 1978). The index of aggregation, K, was also calculated as

$$K = x/(S^2-x)$$

to indicate the extent of aggregation of the eggs

(Southwood, 1978). This experiment was repeated three times with nine replicates each.

4.3 RESULTS

4.3.1 Oviposition response on host, non-host and wild host plants (choice test)

Fertile eggs were deposited on all three plants types presented in plant choice tests. However, more eggs were deposited on cowpea, the host plant (Table 1). Eggs were laid singly as well as in batches of 2-16 eggs (Plate 9). As shown in Fig 3 gravid *M. testulalis* females can lay their eggs over a period of 18 days but the maximum number of eggs were laid on the seventh to the eighth day. Observations from ten replicates showed that the mean numbers of eggs deposited on cowpea, cotton and sunhemp were 102 $\pm$ 53, 51 $\pm$ 19 and 40 $\pm$ 12 respectively. There was statistical significant difference between days but none between the plants at P=0.05 (Appendix 3). Analysis of the data showed that eggs distribution was as follows: cowpea 40%, cotton 19% sunhemp 12% and pots 29%.

4.3.2 Effect of photoperiodism

No eggs were laid during the light period i.e. between 0700 and 1700 hours. Egg-laying started about two

Table 1:

Oviposition response in three-way choice test (n=10)

	Cowpea	Cotton	*Crotalaria*
Plant	783	364	242
Pot	239	155	154
Total	1022	519	396
Mean ± S.E	102 ± 53	52 ± 19	40 ± 12
%Total	52.76	26.79	20.44
%Plant only	40.42	18.79	12.49

Table 2:

Number of eggs per oviposition bout

(one observation lasting six hours)

Bout	Duration (min)	No.of eggs deposited per unit area of surface ($\times 10^{-3}$ cm^2)		
		On leaf	On cage	%Total
1st bout	8	360	56	28
2nd bout	13	823	150	65
3rd bout	3	103	7	7

Av. area of leaf surface = 19.44cm^2
Av. area of cage = 306cm^2

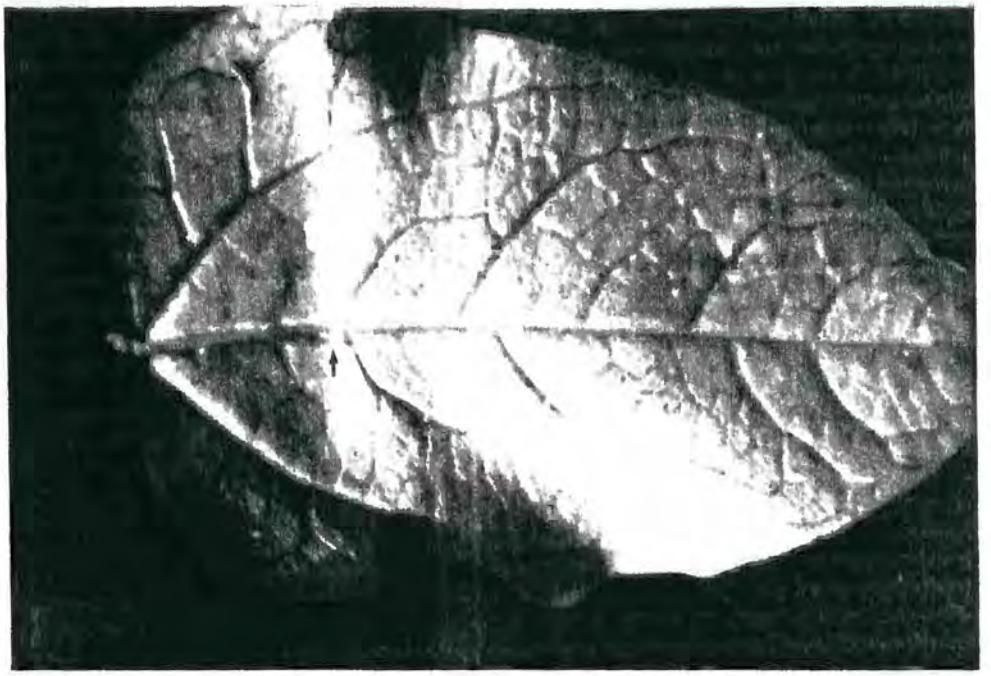


Plate 9: Eggs of M. testulalis on cowpea leaf (arrow).

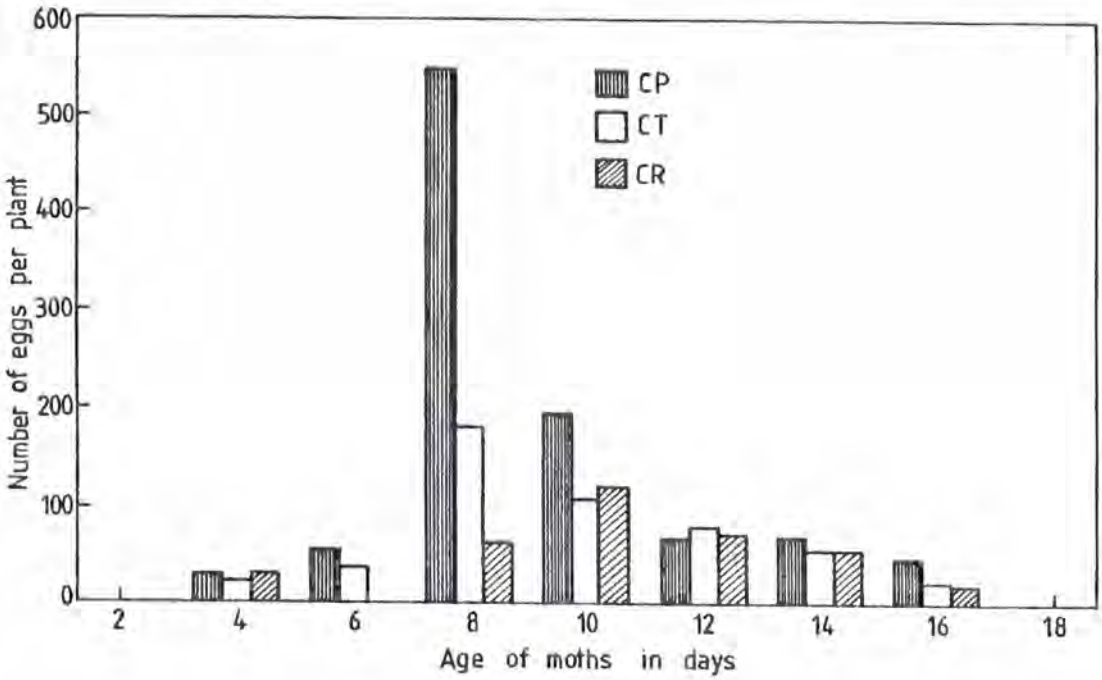


Fig. 3. Number of eggs deposited per day on the plants (CP-Cowpea, CT-Cotton, CR-Crotalaria)

hours after lights-off, at which time about 20% of the total number of eggs were laid. Peak oviposition, about 60% of the eggs laid was recorded between 2200 and 2400 hours (Fig.4), i.e six hours after lights-off. This gave some idea as to when observations for further experiments were to be carried out. After 0600 hours no more eggs were deposited. With respect to the age of moths, it was found that moths' oviposition was at its peak on the fourth day after mating (Fig.5).

4.3.3 Sequence of events leading to egg deposition

Laboratory observations

Moths exhibited several behavioural events repeatedly. Such events included flight, walking, sitting or pausing, ovipositing and occasional feeding (Plate 10). The antennae were continously waving all through the period of observation except when they were being cleaned or groomed. The basic pattern and sequence of the oviposition process is shown on the ethogram on Fig.6. Mated females started oviposition activities approximately 1.6hr. after dark. At the onset of darkness, the moth was observed sitting quietly while waving the antennae held laterally. Sometimes letting the antennae touch the surface of the cage in a slow tapping/drumming fashion. She then flew either to another part of the cage or the plant in the cage. She then bent her abdomen downwards,

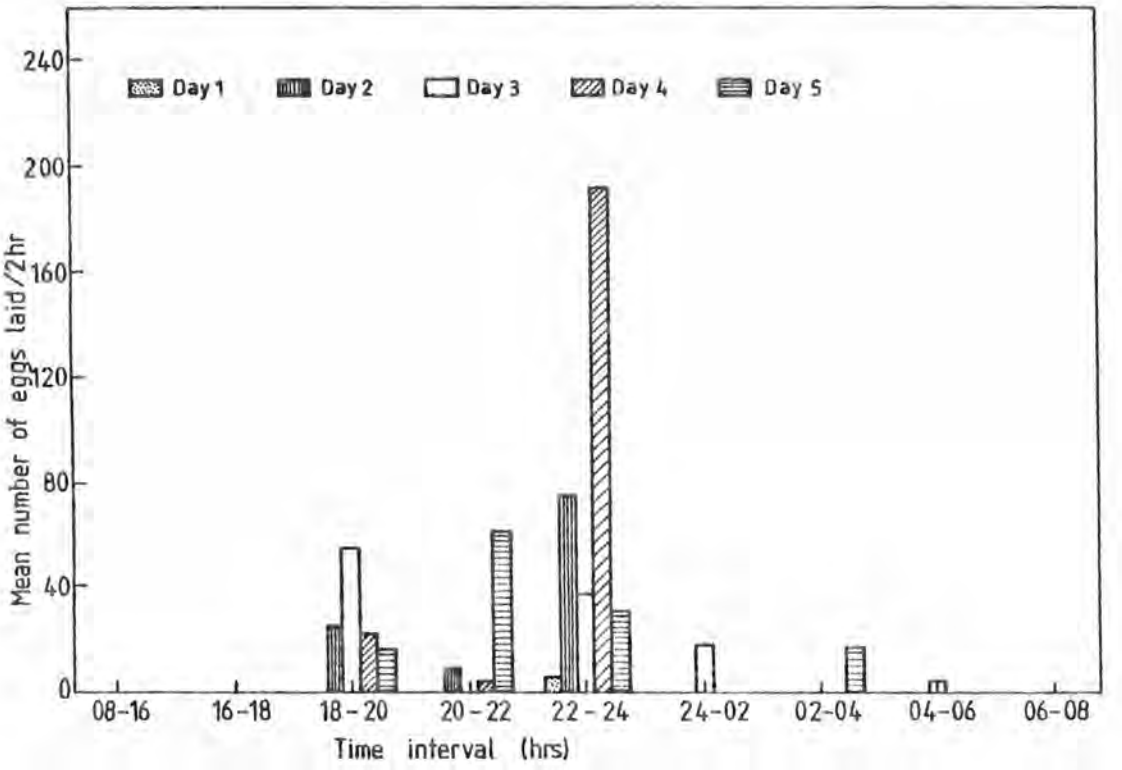


Fig. 4. Egg deposition at different hours during the 5-day oviposition observation period

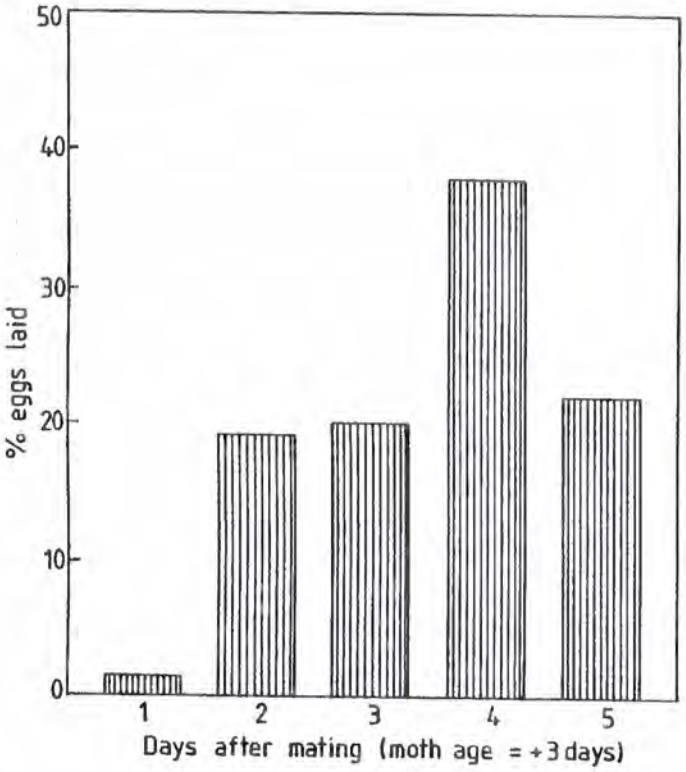


Fig. 5. Percent eggs laid per day after mating

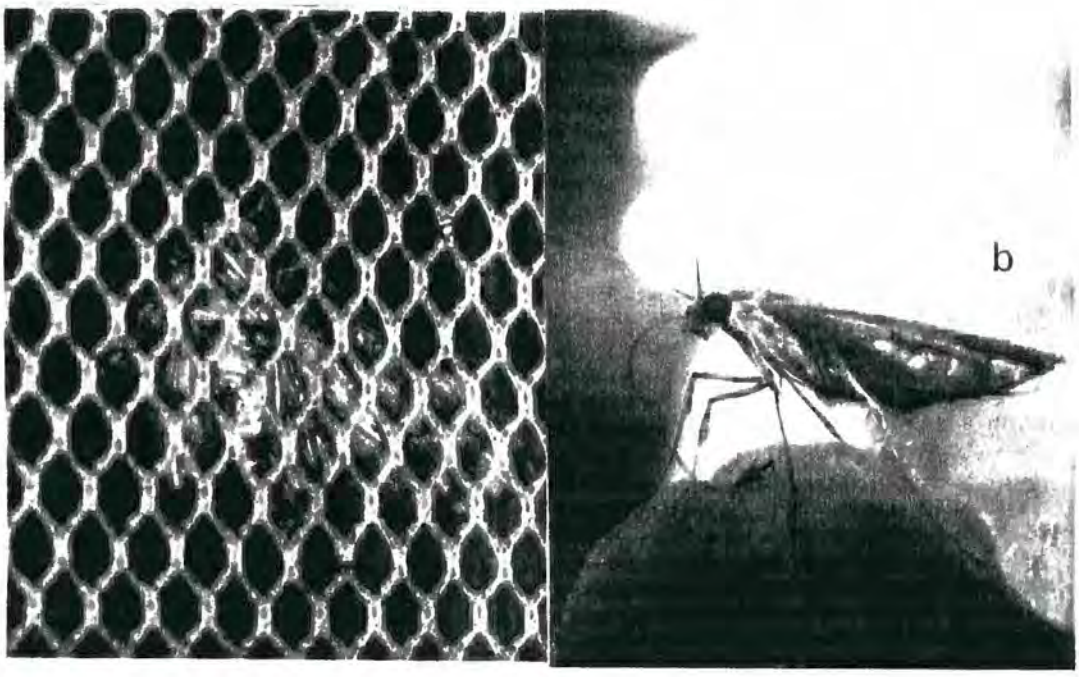


Plate 10: (a) M.testulalis female bending abdomen during a bout of egg deposition (arrow=newly deposited egg), (b) feeding on sucrose (arrow=proboscis sucking sugar solution from cottonwool).

sometimes dragged the ovipositor tip along the surface while walking. The moth paused and made further extensions of the ovipositor as it scanned the immediate surface. This was followed by egg deposition. Then she immediately flew away and landed on another area where she repeated the same sequence of events as shown in Fig.6. After going through an oviposition bout lasting 6 to 18 minutes (mean = 9 $\pm$ 2.87 min) and laying at least four eggs (about 7% of the total number of eggs laid in the three bouts) (Fig.7 and Table 2), a period of rest followed. During the six hours observation period an average of three oviposition bouts were executed by each moth examined. More eggs were laid during the second bout. During the rest period the moth sat quietly waving her antennae. She also walked about, took short flights and groomed the antennae with the forelegs. Non-ovipositing periods were longer than actual ovipositing periods (Fig.7). The frequency of the various events has been quantified and is presented on Table 3. Sitting or resting periods were observed to occur more often and for longer periods, followed by flight and walking events. During these observations moths were seen to deposit more eggs on the cage surface than on the leaves but comparing per unit area of the surfaces, more eggs were laid on the leaves than on the cage per cm^2 (Table 4).

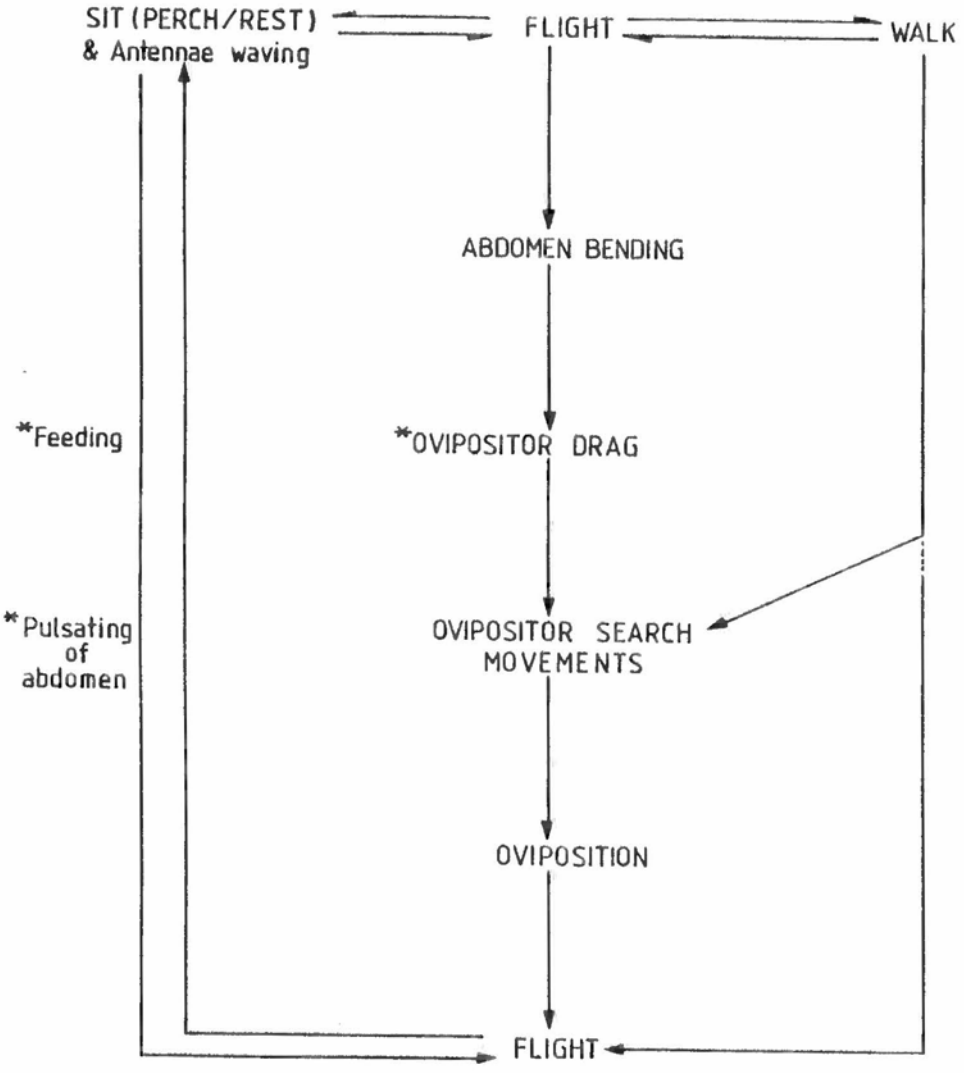


Fig. 6. Flow chart showing sequence of events associated with oviposition behaviour in M. testulalis. *Occasional activity

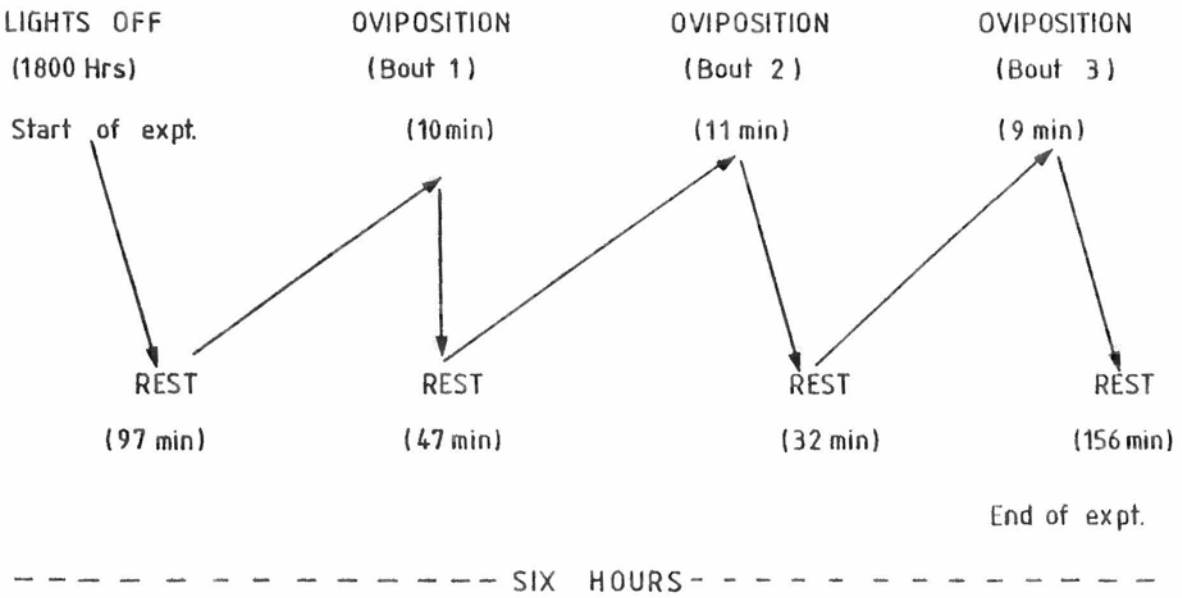


Fig. 7. Oviposition Bouts and Rests periods

Table 3:

Frequency of oviposition behaviour events and their duration over a 6-hr observation period

	Behaviours	Frequency	Duration (min.)
1)	Sit	15	21
2)	Flight	12	2
3)	Walk	10	1
4)	Oviposition Search + Oviposition	7	2
5)	Antennae Tapping	5	-
6)	Wing Twitch	3	-
7)	Ovipositor Drag (Defaecation)	2	2
8)	Grooming (Antennae)	2	-
9)	Feeding	1	4

On the non-host plant, cotton, moth was observed to exhibit jerky movements. Antennae waving was very vigorous. In one observation, host plant, cowpea was re-introduced and the visible change in behaviour of the moth was remarkable. The jerky movements exhibited by the moth when cotton was in the cage ceased as cowpea replaced the cotton. The moth resumed egg-laying behaviour and the antennae waving was not as vigorous as when cotton was in the cage.

The antennae and the ovipositor were the main parts of the moths' body involved in the selection of oviposition site. The ovipositor was especially used at the final stage when eggs were just about to be deposited; the ovipositor tip scanned the surface prior to depositing each egg. During actual deposition of an egg, when the ovipositor tip was in direct contact with the site of oviposition, the antennae no longer made contact with the substrate but was held upwards and slightly lateral or parallel to the substrate. Only the ovipositor tip scanned or searched the immediate oviposition site/surface.

Field Observations

At the beginning of the field studies, commencing at 1800 hours, it was observed that moths were perched at positions on which they had been sitting all through the

day. Moths generally became active when darkness set in and under field conditions this occurred at 1920 hours. Moth movements observed at this hour were vigorous antennae waving accompanied later by flight activity. Other events exhibited by moths were sit (perch), walk and abdomen bending which in most of the cases was associated with deposition of eggs. The durations and frequency of these events observed in the field are given on Table 5. Oviposition activities were minimal and commenced at about 2140 hours each day. Flying, perching/rest and walking events were more frequent. Sequence of events leading to egg deposition were similar in pattern to that observed during the laboratory studies. (see Fig 6). Flight always followed abdomen bending/scanning the surface with ovipositor tip, and in about 60% of such manoeuvres, eggs were deposited.

In the second set of experiments which involved various combinations of host and non-host plants per cage; the moths were active from 1926 hours each day in all the cages. Moths in cage 1 (containing only the non host plants) were not as active as those in cages 2 and 3 where host plant of the moth was present. The mean number of eggs deposited in the three cages of the two-choice combinations consisting of host and non-host plants is presented on Table 6. The moths deposited more eggs on the host plant -only-combinations i.e cage 3, than in the

Table 4:

Total number of eggs deposited per surface

Observation	No. of eggs deposited per unit area (x $10^{-2}cm^2$)	
	On leaf	On cage
1	72	16.7
2	0	13.1
3	0	4.9
4	128.6	21.2
Total	200.6	55.9
Mean	50.1 ± 31.1	14.0 ± 3.4

Table 5:

Behavioural events observed in the field and their duration

Event	Duration (min)		Frequency
	Total	Mean ± S.E	
Sit	489.97	5.09 ± 1.29	98
Flight	38.58	0.42 ± 0.07	92
Walk	5.58	0.56 ± 0.28	10
Abdomen Bend	-	-	1

other cages. Similar to observations made in the laboratory, the antennae and the ovipositor were also observed in the field, to be involved in the oviposition activities.

4.3.4 Pattern of egg distribution on cages and leaves

The mean number of eggs and the variance per unit of 3 by 2cm were obtained to determine the distribution pattern of the eggs of *M. testulalis* on natural and artificial oviposition surfaces. The mean was found to be less than the variance ($x < s^2$) in all the three sets of experiment (Table 7) for both the leaves and the cages. This implies that the dispersion of eggs on the cages and leaves is contagious, that is, the presence of an egg in a cell increases the chance of another egg being laid in that particular cell (Southwood, 1978). Hence the eggs seem to be in groups or batches. The dispersion index (I_D) and the index of aggregation (K) are given on Table 7. All the values of I_D obtained were found to be significant at P= 0.01, hence the distribution of eggs on cages and leaves was not random (Southwood, 1978). This results also imply that the eggs are not randomly laid on the cages or leaves, but rather seem to aggregate (Southwood, 1978). This agrees with the earlier conclusion from the means and variances of eggs per cell. The value of K for most of the cages was less than unity

Table 6:

Mean number of eggs deposited on the various plant combinations

Plts combtn	*Mean egg Nos./cage ± S.E.
Cage 1 (A + A)	1.2 ± 1.2b
Cage 2 (A + B)	0.8 ± 0.4ab
Cage 3 (B + B)	18.4 ± 11.4a

+A =Cotton (Non-host plant)
+B =Cowpea (Hostplant)
*Means with the same letters are not significantly different at P=0.05.

Table 7:

Spatial analysis of eggs distribution by M. testulalis on cages and leaf

Set	N	X	S^2	I_D	K
+1	51	2.41	53.53	1110.58	0.11
+2	51	0.45	6.05	672.22	0.04
+3	51	1.55	36.57	1179.68	0.07
*4	56	4.52	40.04	487.21	0.57

+Cage; *Leaf.
Chi-squared value (X^2) at 50 d.f and 5% level= 67.50
Chi-squared value (X^2) at 50 d.f and 1% level= 76.15

and this indicates a very high degree of aggregation of the eggs (Southwood, 1978).

4.4 DISCUSSION

In the experiments with the three test plants, *M. testulalis* deposited eggs on cotton and *Crotalaria* in spite of the presence of cowpea, its host plant. More eggs were deposited on cotton than on the wild host plant which had been previously reported to attract more eggs than the host plant. The observation that *Crotalaria* plants did not attract strong oviposition responses as cowpea and cotton is a bit surprising because studies by Jackai and Singh (1981, 1983) had shown *C. juncea* was better than cowpea in attracting eggs from ovipositing *M. testulalis*. This contradiction may be due to difference in experimental conditions including the difference in geographical location of the studies. Since cotton is a non-host, observations reported here raised some questions as to what characteristics of the cotton plant attracted some eggs.

Many activities of insects show some daily periodicity. This has been observed in feeding activities and even in emergence patterns of insects. For instance, the potato moth, *Leptinotarsa decimlineata,* exhibited a daily rhythm of feeding activity in response to diurnal changes in light (Wigglesworth, 1965), while the noctuid moths, *Busseola fusca* and *Sesamia calamistis* showed an emergence rhythm in which moths emerged after sunset or

soon after placing mature pupae in darkness (Usua, 1970). In the present studies, it was observed that the oviposition activities of *M. testulalis* showed diurnal periodicity. Both in the field and the laboratory, moths were observed to begin oviposition activities at particular hours of the day. This was at two hours after lights-off in the laboratory, peak oviposition occurring between six and seven hours after dark. Onset of darkness, from 1920 hours, stimulated flight activity. Oviposition activities were noticed from 2100 hours up to 0100 hours. This supports the observations by earlier workers, that *M. testulalis* executes or performs most activities, in this case ovipositon, from dusk to just before dawn. *M. testulalis* gravid moths were observed to fly actively from thirty minutes after lights-off up to two hours later, before oviposition started. This initial active flying of the gravid moths, observed just after dusk could be foraging flight reported in some other moths such as *Manduca sexta* (Yamamoto *et al.*,1969), *Etiella zinckenella* (Hattori, 1986) and *Heliothis virenscens* (Ramaswamy, 1988).

M. testulalis activity patterns observed in the current studies appear to concur with what has been reported by other workers. Akinfenwa (1975) observed that *M. testulalis* adults were caught between 2000 and 0045 hours using ultra-violet lamp traps with peak flight

activities occurring between 2000 and 2100 hours. These periods of moth activity are similar to those reported in this work. The effect of photoperiod on egg-laying has been reported in other moths by various workers (Lum and Flaherty, 1970; Kumar and Saxena, 1985a; Singh and Rembold, 1989). It was found that there was a definite day-night rhythm of oviposition, and in all cases maximum number of eggs were deposited in the dark period. It had been earlier reported that larval activities and adult emergence of *M. testulalis* showed diurnal rhythm of activity, and that larvae were nocturnal being active throughout the night while adult emergence was highest early in the night (Usua and Singh, 1979; Okeyo-Owuor and Ochieng, 1981).

The pausing accompanied by ovipositor extension and its scanning movements on the surface preceeding the deposition of egg by the ovipositing moth as reported in this work, has been reported in other insects (Chadha and Roome, 1980; Hattori, 1986, 1988; Stoffolano and Yin, 1987). The ovipositor tip is equipped with sensory hairs which assesses the suitability of the oviposition substrate. In *C. partellus*, it was suggested that these hairs at the tip of the ovipositor may act as a final defence against laying of eggs on chemically unacceptable surfaces which may have missed assessment by the antennae and tarsal sensilla (Ogwaro, 1978; Chadha and Roome, 1980;

Alghali, 1984; Hattori, 1986, 1988; Ramaswamy, 1988, 1990).

M. testulalis exhibited long periods of rest interspersed with flight and walk activities. Such flights included movement from plant to plant and to cage surface, between oviposition bouts. Several short flights without oviposition occurred towards dawn especially in the field observations. Similar behaviour has been noticed in *Heliothis* spp (Topper, 1987; and Ramaswamy, 1990). *H.virescens* females exhibited major periods of flight just before and after sunset and sunup (Ramaswamy, 1990). Topper (1987) also reported such flight activity in *H. armigera* shortly before and after sunset. It was found that such flight resulted in a redistribution of moths in the field and crops, for flight seen in the early morning hours led to the finding of a suitable cover where moths spent the daytime period.

In *M. testulalis* three oviposition bouts lasting 6-18 minutes were recorded and there was no more egg-laying by 0600 hours. The female distinctly exhibited active periods during which the three bouts of oviposition interspersed by flight and resting occurred; feeding was rare although noticed in two observations. This suggests that in cowpea fields gravid moths may move around from plant to plant or leaf to leaf and oviposit several eggs

at each visit; although it is not clear how widely they may move during a night. Nocturnal periodicity is an important survival cum dispersal adaptation which protects the moths from predators while searching for host plants for oviposition (Singh and Rembold, 1989). In addition freshly laid eggs are protected from desiccation especially being in the tropics, unlike if the eggs are deposited in the daytime.

The pattern of egg distribution in the present studies was found to be contagious. The term contagious is used to describe a population of individuals which tend to occur in clumps or aggregates (Stiteler and Patil, 1971; Southwood, 1978; Kao, 1984). The results showed that the presence of an egg in a unit/site increased the chance of another egg being laid in that particular unit/site. The eggs are therefore in clusters (groups or batches) but scattered around the substrate. The number of eggs oviposited by gravid females of *M. testulalis* at each area or site varied from 1 to 16. However, more often one or two eggs were deposited at each site. Oviposition behaviour among moths varies quite a lot. For example, *E. zinckenella* exhibits oviposition bout behaviour but is reported to deposit 1 to 8 in a bout and each egg is placed at a different site (Hattori 1986, 1988). Kobayashi (1960) reported in *Pieris rapae crucivora* such oviposition behaviour resulting in a

concentric distribution of eggs in a cabbage farm. For *M. testulalis* it appears that the aggregation of the eggs seem to be due to the active behavioural process of the moths rather than the environment.

CHAPTER 5

MORPHOLOGY OF SENSORY ORGANS ASSOCIATED WITH OVIPOSITION ACTIVITIES IN M. TESTULALIS

5.1 INTRODUCTION

There is limited information on the ovipositor and antennal sensilla of *M. testulalis*. The purpose of this work was to examine the ovipositor and antennae of this moth, and thereby identify the types, numbers and distribution of sensilla on these organs.

5.2 MATERIALS AND METHODS

5.2.1 Light microscopy:

Specimens for light microscopy studies were prepared using two different methods. In the first method, the antennae and ovipositors were fixed in 10% formal saline (0.9gm of sodium chloride + 10ml of formaldehyde made to 100ml with double distilled water). The specimens were left in the fixative for two days, after which they were rinsed three times with distilled water, each rinse lasting 10-15 minutes using a shaker (Thomas rotating

apparatus). This was followed by dehydrating the tissues with ascending concentrations of ethanol. The specimens were cleared for two days by passing them through two changes of cellodin methyl benzoate. The cleared tissues were mounted on glass slides using glycerin jelly. In the second method, the antennae and ovipositors were boiled for 15 minutes in 10% potassium hydroxide solution in a water bath. They were mounted with Hoyer's solution and examined under a Leitz Dialux 22EB light microscope. The number and dimensions of the antennal segments were recorded from these specimens. Measurements of the segments were made with the aid of an eye piece graticle and a microscope stage micrometer.

5.2.2 Scanning electron-microscopy:

Female *M. testulalis* moths were imobilized with carbon dioxide, and then their thorax was crushed with a pair of forceps. The heads, with the antennae still attached, and the ovipositors were removed and fixed in 2.5% glutaldehyde for 48 hours. These specimens were then dehydrated using ascending grades of alcohol (30%, 50%, 70%, 80%, 90%, 96% and 100%) for forty-five minutes per alcohol grade. They were in turn air-dried and each specimen was mounted on specimen stub using a double-sided tape. Silver paint was applied around the specimen to improve specimen contact with stub. The specimens were

then coated with gold in a Jeol JFC-1100E fine coat ion sputter and examined using Jeol JSM-T330A scanning electron microscope. The number, measurements and description of the various sensilla types were obtained from the scanning electron micrographs taken.

5.3 RESULTS

5.3.1 Antennal morphology:

M. testulalis have an average of 78 antennal segments consisting of a scape, pedicel and a 75 to 76-segmented flagellum (Table 8). The antennae are filiform in male and female moths, and the mean number of segments are about the same in both sexes. The antennae measures 11.5 mm on the average (10.5-13.0 mm). The whole antennal surface is reticulate (net-like scuplture) terminating at the distal tip with two to three finger-like structures (Plate 11 (2)). The first two proximal segments, i.e the scape and pedicel are larger than the flagellar segments. The mean length and width of the scape is 208 x 292um respectively, the pedicel 121 x 179um while the proximal or first flagellar segment (annular) is 100 x 147um and the apical or terminal segment is 96 x 41um. The segments from the proximal part of the antennae are shorter and wider than the distal segments which are longer and narrow resulting in the filiform shape of the antennae. Segments

Table 8:

Antennal measurements of M. testulalis

Segments	N	Length(um)	Width(um)
Terminal	6	96.0 ± 2.1 (88-104)	41.3 ± 1.3 (40-48)
Proximal	5	100.8 ± 5.4 (88-120)	147.2 ± 1.9 (144-152)
Pedicel	5	121.6 ± 9.2 (96-152)	179.2 ± 3.1 (168-184)
Scape	5	208 ± 5.6 (192-224)	292.8 ± 3.1 (288-304)

1 to 26 from the base of the antennae, are 120-152um while the middle segments are 56-120um and the distal segments, from segments 52, are 40 to 56um, widthwise. The lengths varied between 80-104um for the first 17 basal segments, 104-152um for the middle 44 segments and 96-160um for the distal 26 segments.

Antennal sensilla of M. testulalis:

A number of different types of sensilla were seen on the antennae and can be distinguished by their size, surface sculpture and the manner of protrusion from sockets and pits on the antennal surface (Plate 11). About seven types of sensilla, labelled a to g, were found on the flagellum of the antenna. Most of them were located on the ventral scaleless surface of the flagellum. Sensilla on the whole antennae were examined but the description of the types given below was put together from light and scanning electron microscope examination of the first four terminal antennal segments.

Type a: [Plate 11 (1 and 3)]

These sensilla were present on each of the four apical segments examined. They were located on the ventral side of the flagellum, towards the proximal end of each segment. They arise from distinct round cuticular sockets which are about 2um high from the antennal surface. The surface of the sensilla is covered with fine

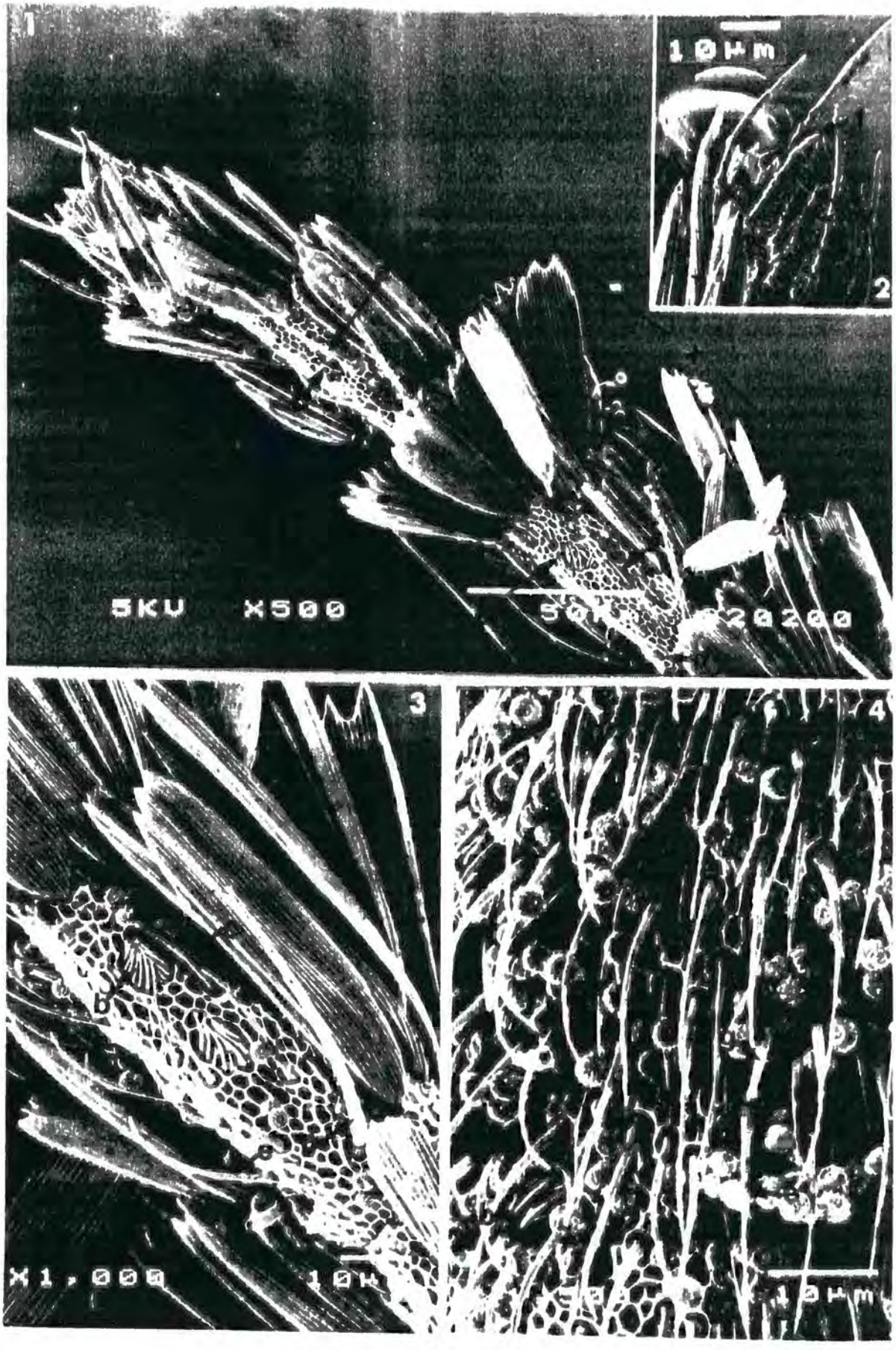
Plate 11: Scanning electron micrographs of M.testulalis antennae:

(1) Two terminal segments showing types a-e sensilla; x delineates a single segment.

(2) Fingerlike structures (y) at tip of apical segment bearing sensilla stylocinica (type f sensilla).

(3) Enlarged (4th) distal segment.

(4) A distal segment bearing several basiconica sensilla (type g).


1
5KV X500
2
10µm
3
X1,000 10µ
4
10µm

ridges giving it an almost smooth appearance. The shaft is about 40.7um long and the basal width 2.3um. The shaft tapers into a blunt tip.

Type b: [Plate 11 (1, 3, and 4)]

These were small sensilla sunk below the antennal surface. Each sensilla was surrounded by a fringe of 10 to 14 toothlike structures or microtrichia which were about 2.6-6.7um in length, projecting from the edge of the pit bearing the sunken peg. These groups of sensilla appeared to increase in numbers towards the proximal segments of the flagellum. For instance, segments 1 to 3 had one each while segment 4 had at least two, placed one behind the other (Plate 11 (3)). In the basal or proximal segments, there were many sensilla of this type per segment.

Type c: [Plate 11 (1 and 3)]

These sensilla are about 12-15um long and 2um in basal width. They have deep middle grooves running longitudinally. They arise from cuticular pits and their tips appear blunt. They were seen on most of the other segments except on the apical or terminal segment. They are more of less ventro-laterally placed.

Type d: [Plate 11 (1)]

On the second segment were observed two small

sensilla measuring 5.4um with a basal width of 1.4um. They project almost perpendicular to the antennal cuticular surface from cuticular pits.

Type e: [Plate 11(1, 3, and 4)]

These are the most numerous sensilla and are of different lengths. The lengths vary between 18.8 and 56.3um with basal width of 1.5-2um. The sensilla arise from cuticular pits on the flagellum and are curved towards the distal end of the antenna. Their surfaces are covered with annular ridges, especially towards the tapering tip, while the basal portion of the sensilla shaft shows longitudinal grooves. These sensilla are located all over the surface of the flagellum and are found on all the segments, with the highest density on the proximal segments and scaleless areas of the flagellum.

Type f: [Plate 11 (2)]

This was noticed on the terminal segment, right at the tip of the fingerlike projection. It has a base of about 2.8um which tapers to 2.5um with a length of 1.9um. A pointed projection of about 3um long and 1.2um at the base tapering to 0.6um at the tip, arises from this base

Type g: [Plate 11 (4)] These are stout looking sensilla and arise from cuticular pits among the type e sensilla. A pair of these sensilla were noticed on the basal

segment. They are about 11.7um in length and 1.4um at the base with blunt tips.

5.3.2 Ovipositor morphology:

The ovipositor of *M. testulalis* is retractile and lies within the seventh abdominal segment. It is tube-like with a hairy tip. The ovipositor tip is oval and consists of two valves or lobes which are covered with a large number of closely spaced hairs or sensilla approximately 236 in number. These hairs can be grouped into two main types according to the surface sculpture of the shaft (Plate 12) and the length of sensilla. The surface of the hair shaft are either covered with many microlobes giving the sensilla a saw-like appearance, or transverse/annular grooves.

Sensilla on the ovipositor of M. testulalis:

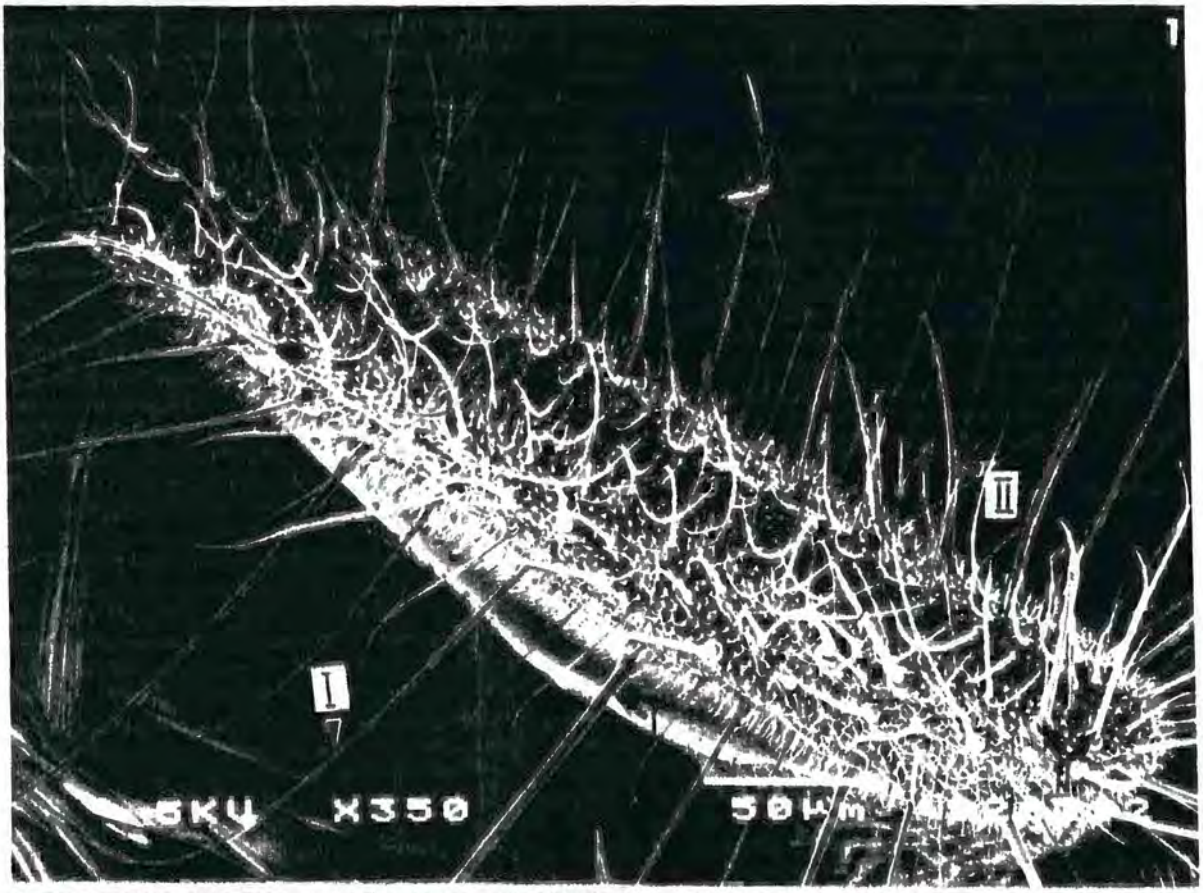
There are two main types of sensilla on the ovipositor tip and are distinguishable on the basis of their surface sculpture. Each of these two types may have sub-types according to their lengths, position on the ovipositor and manner of projection from the ovipositor surface. The rest of the ovipositor surface is covered with numerous microtrichia which are curved downwards towards the ovipositor lobes.

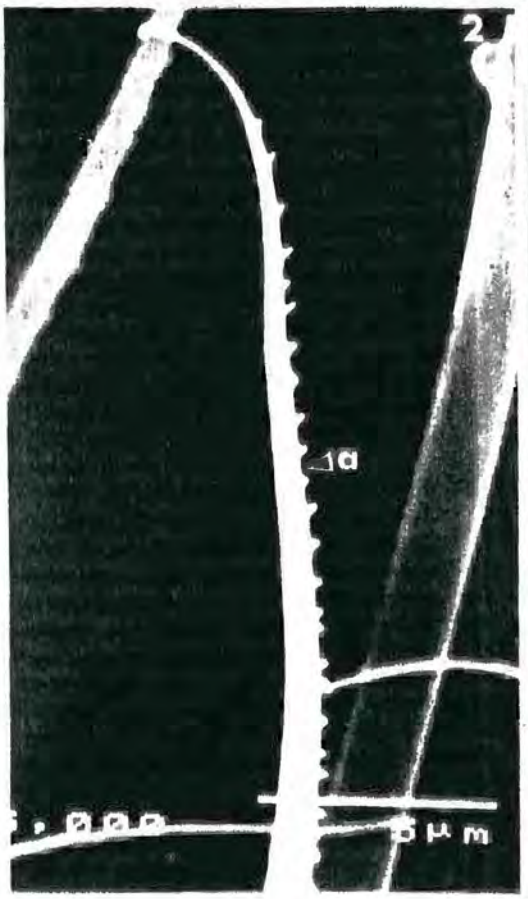
Plate 12: Scanning electron micrographs of M. testulalis ovipositor:

(1) The ovipositor tip showing distribution of sensilla types I and II.

(2) Tip of type I sensilla (a).

(3) Type II sensilla with microtrichia (m) in the background, b & c = robust and slender type II sensilla.


1
I
II
6KV X350
50µm


2
a
5µm


3
m
b
1
5µm

Type I: [Plates 12 (1 and 2)]

These are very numerous and located more at the edge of the ovipositor valves or lobes. Their surfaces appear serrated (sawlike) at the distal end on one face only (other face is smooth), while the basal portions are smooth (Plate 12 (2)). The longer sensilla alternate with shorter ones. The long sensilla are between 170 and 283um in length with a basal width of 2-5um while the short ones are 43-135um long with basal width of 0.6-1um. The sockets from which the shaft of the sensilla arise are tight around the base of the shaft. The sensilla taper into sharp ends, covered on one side with the microlobes.

Type II: [Plate 12 (1 and 3)]

There are seven prominent sensilla of this type on each ovipositor valve. They have a generally smooth-looking surface which shows annular or transverse ridges at close examination (Plate 12 (3)). The sensilla are between 57 and 79um long with a basal width of 3.8um. They taper into a blunt tip. They are few in number compared to the type I sensilla. There are two pairs at the dorsal end, one in the middle half and two towards the hind end of the ovipositor valve. The sockets appear loose around the base of the sensilla.

5.4 DISCUSSION

The antennae

The gross morphology of the antennae of *M. testulalis* seems to be similar to those of some other lepidopteran species described. For instance, the presence of scales on the dorsal part of the antennal segments, and the high density of sensilla on the ventral scaleless part as observed in *M. testulalis*, have been reported in some small ermine moths, *Yponomeuta* species (Van der Pers *et al.*, 1980) and the European sunflower moth, *Homoesoma nebulella* (Faucheux, 1991). Possibly, the sensilla covered by the scales on the dorsal area of the antennae may not play much role during scanning of the oviposition site, but could be involved in sensing the moth's immediate environ and protect the antennae against harsh climatical conditions such as drought and scorching sun.

The seven sensilla types on antennae of female *M. testulalis* were designated a to g. From descriptions given by Van der Pers *et al.* (1980); Anderson and Hallberg (1990); Ritcey and McIver (1990); Faucheux (1990, 1991); and Waladde *et al.* (1990), the sensilla types described above may be identified as:

i) types a and e = sensilla trichodea

ii) type b = sensilla coeloconica

iii)type c = sensilla auricilia (Faucheux, 1991)

iv)types d and g = sensilla basiconica

v)type f = sensilla styloconica.

The types b and e sensilla were many and found on almost all the segments. Type b sensilla are similar to the sensilla coeloconica type I described by Faucheux (1990) on *Agathiphaga vitiensis* (Lepidoptera: Agathiphagidae). A large number of these sensilla were found on the proximal segments compared to their numbers on the distal segments of the flagellum of *M. testulalis*. These sensilla are most likely involved in plant odour perception as found in Agathiphagidae and other insects (Altner and Prillinger, 1980; Zacharuk, 1985; and Faucheux, 1990).

The type e sensilla are trichodea from their ultrastructural morphology and their position on the antennal flagellum suggests strong similarity to some sensilla described in other lepidpteran species whose function was proposed to be pheromone perception (Kaissling, 1971; Steinbrecht, 1973; George and Nagy, 1984; Keil, 1984; and Zacharuk, 1980, 1985) probably through olfactory sensing.

During observations of the oviposition process as reported in chapter 4, female *M. testulalis* moths were seen to scan the oviposition surface with the ventral part of the antennae. Considering their morphology and

position, the type a sensilla of *M. testulalis* are believed to be mechanoreceptors while types d, f and g are probably chemoreceptors responding to odours of food and oviposition site as suggested by Faucheux (1990). The sensilla types d and g of *M. testulalis* are morphologically similar to sensilla basiconica described by Faucheux (1990) and other authors on some lepidopteran species, and type f sensilla, which was noticed on the apical part of antennae, resemble those styloconica sensilla described by Van der Pers (1981) and other authors. Van der pers (1981) referred to them as also responsible for plant odour perception in *Yponomeuta species* and *Adoxophyes orana*. However, electrophysiological and further behavioural studies are necessary to ascertain similarity in function of these sensilla in *M. testulalis*.

The Ovipositor

The ovipositor of the legume pod borer, *M. testulalis*, was found to have a large number of sensilla on each ovipositor valve or lobe, which have been classified for easy reference, as type I and type II according to their structural morphology. The density of the type I sensilla found on the ovipositor tip suggest that these sensilla may be involved in mechanoreception while the type II sensilla may act as chemoreceptors (Chadha and Roome, 1980; Valencia and Rice, 1982;

Faucheux, 1991). In other experiments, it was observed that gravid *M. testulalis* moths preferred rough surfaces to smooth ones as oviposition sites. This confirms the importance of mechanoreceptors in assessing the suitability of the physical nature of the oviposition substrate during oviposition.

The relatively few type II sensilla found among the type I mechanosensilla, are possibly contact chemosensilla and may also serve in preventing oviposition on chemically harmful oviposition surfaces as suggested by Chadha and Roome (1980) in connection with type c chemoreceptors found on the ovipositors of *Chilo partellus and Spodoptera littoralis*, which are morphologically similar. Contact chemoreception has been found to play an important role in the behavioural sequence leading to oviposition in a number of phytophagous insects (Ma and Schoonhoven, 1973; Stadler, 1978; Saxena and Goyal, 1978; Behan and schoonhoven, 1978; and chadha and Roome, 1980). Chadha and Roome (1980), recorded three main types of sensilla which were designated types a, b, and c according to their length, surface sculpture and silver nitrate staining, on the ovipositors of C. partellus and S. littoralis.

The presence of chemosensilla on the ovipositor of *M. testulalis* opens up the question of whether the chemical nature of the oviposition substrate may influence egg

deposition in this moth. However, the large number of mechanosensilla (type I sensilla) and the results obtained in chapter 7 suggests that texture may be more influencial in egg-deposition than chemical, especially towards the final stages of oviposition.

The findings in this work appear to suggest strongly that the various sensilla, both on the antennae and ovipositor of *M. testulalis*, are all most likely brought into play in the oviposition sequence, starting with antennal olfactory responses, followed by tarsal gustatory testing and concluding with a final check by the ovipositor sensilla, especially the contact chemoreceptors. This seems to be the general situation in most Lepidoptera as evidenced by similar accounts reported in *C. partellus and S. littoralis* (Chadha and Roome, 1980) and in *Heliothis punctizer* Wall. (Valencia and Rice, 1982).

Further work, such as transmission electron microscopic studies, is however, needed to characterize the electrophysiology and behavioural role of the ovipositor and antennal sensilla of *M. testulalis*.

CHAPTER 6

FACTORS AFFECTING OVIPOSITION BEHAVIOUR OF M.TESTULALIS

6.1 INTRODUCTION

Factors such as moisture, texture, plant odour/ semiochemicals--behaviour-modifying chemicals (Lewis and Nordlurd, 1985) have been reported to affect insect oviposition behaviour (Ichinose and Honda, 1978; Hattori, 1988; Rembold, 1988; Bierbaum and Bush, 1990; Stadler and Schoni, 1990). Information about such factors and how they influence oviposition in *M. testulalis* can be useful as a data base which can be used in understanding the behaviour patterns of *M.testulalis*. With this idea in mind, experiments were carried out to investigate the effect of moisture, texture, host and nonhost plant odours, as well as extracts, on the oviposition behaviour of *M. testulalis*.

6.2 MATERIALS AND METHODS

6.2.1 Effect of moisture

Seven-day old mated female moths were placed individually in 20 x 6 x 10 cm perspex cages (Plate 13). They were presented with a choice of moist filter paper at one end of the cage, dry filter paper at the other end (Plate 13) and 10% sucrose solution applied on cotton wool as a food source. The moisture on the moist filter paper was maintained by allowing one end of the filter paper to be in touch with water contained in a pocket made out of aluminium foil (Plate 13). The dry filter paper was also placed in a pocket of aluminium foil paper for uniformity but there was no water in the pocket. After 24 hours each paper was examined for eggs under a Wild M8 dissecting microscope and the number of eggs laid on each paper was recorded. The experiment had 30 replicates. Data collected was transformed into logarithms of square-roots and subjected to Student's T-test.

6.2.2 Effect of oviposition substrate surface texture

One side of a six sided perspex cage measuring 6 x 3 x 15 cm was covered with mosquito netting material to provide a rough surface while the remaining five sides were left uncovered. Seven-day old mated female moths

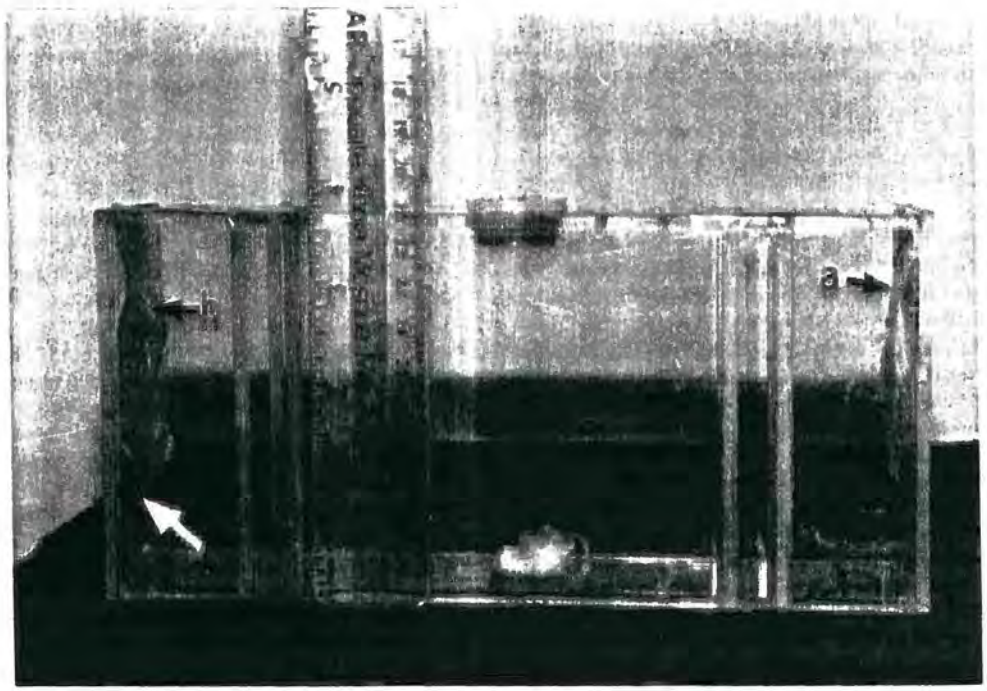
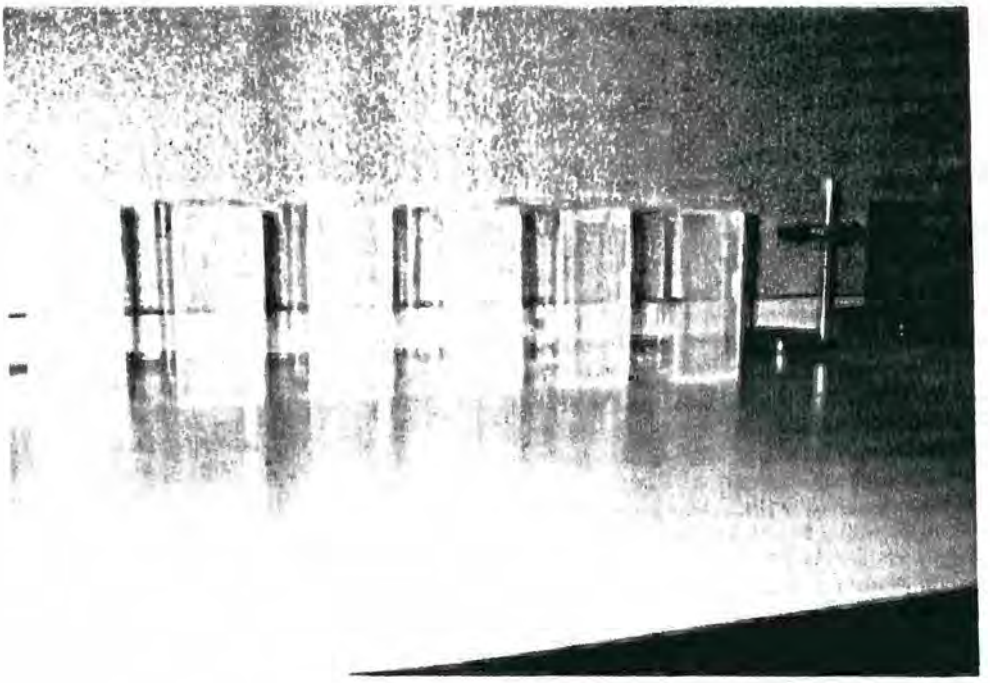


PLate 13: Set-up to examine effect of moisture on oviposition of M. testulalis; a=dry filter paper, b=moist filter paper, c=aluminium foil paper pocket.

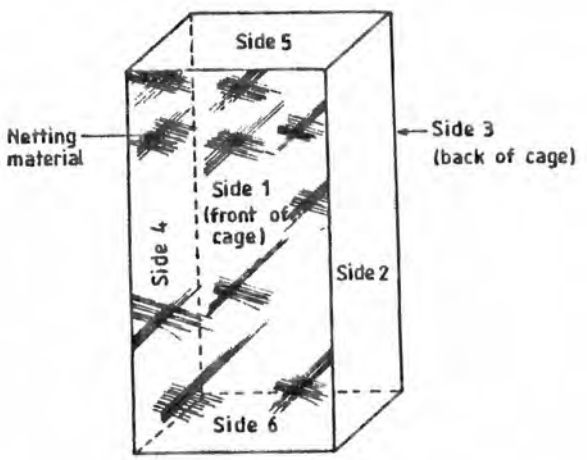
were placed individually in the cage and were provided with a cotton wool pad soaked with a 10% sucrose solution (Plate 14). Each cage was an experimental unit and the experiment had six replicates. Every twenty-four hours, the number of eggs laid on the six sides of the cages were counted and recorded. This was done over a three days period. The egg counts were then standardized to counts per unit area. The data was then transformed into logarithms and subjected to analysis of variance (ANOVA) with means separated by the Student-Newman-Keuls' test.

6.2.3 Effect of plant odour

Seven day old, mated female moths were placed in the middle compartment of a 20 x 6 x 10 cm perspex cage. Cowpea leaves were introduced into the right-side compartment while the left compartment was left empty (Fig 8). Each of the two outer compartments was separated from the middle one by a perforated removable perspex sheet which allowed free movement of plant's odour to pass from the right-side-compartment to the compartment containing the moths. Every 24 hours the moth was transferred to a new cage arranged in a similar manner and the number of eggs in the previous cage was recorded. The experiment was run for three consecutive days. Control cages without plants were also set to run concurrently with the experimental cages. This experiment was repeated while



i



ii

Plate: 14. (i) Cages as set to examine effect of texture in M.testulalis (arrow - netting material fixed to side 1). (ii) Sketch of the cage showing six sides numbered

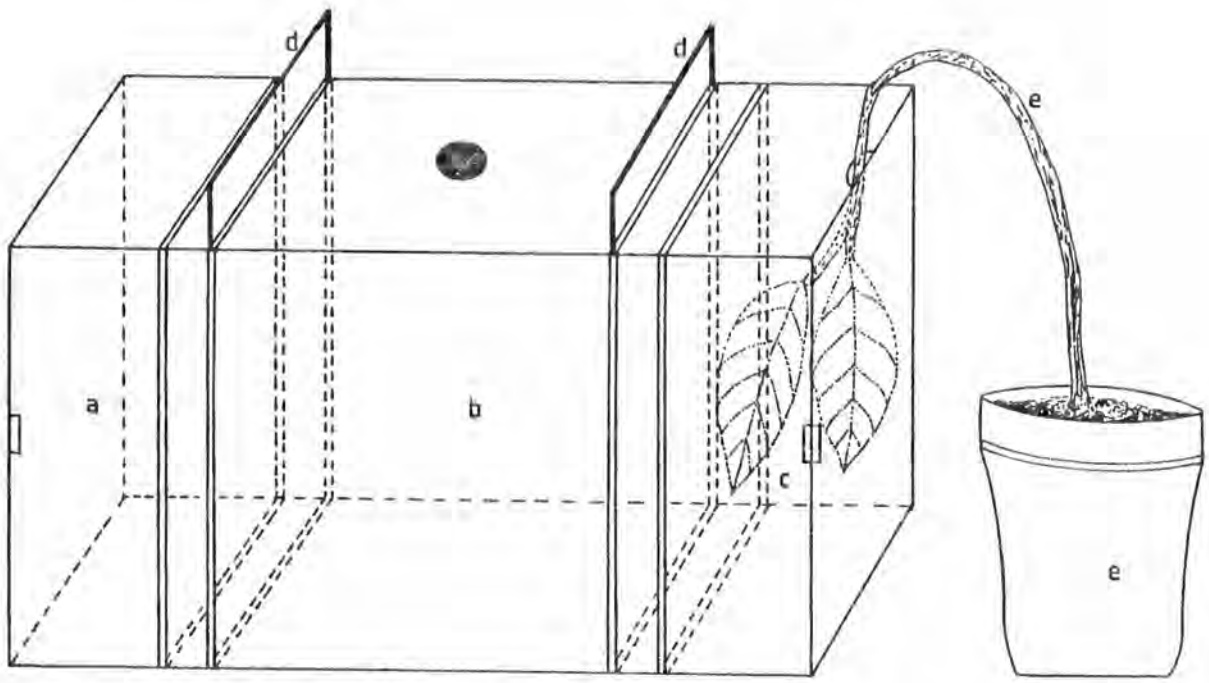


Fig. 8. Set-up for testing the effect of odour on M. testulalis (a, b and c - the three compartments separated by d- perforated sheet fitted into slots, e- potted plant introduced through a slit in the opening).

using cotton and sunhemp as the test plants. Each experiment was replicated ten times.

The experiment was repeated using cowpea while a vacuum pump connected to the experimental cages was used to remove air from the cages thus preventing the building up of plant odour in the cages (Plate 15). In this case, the plant odour concentration gradient was biased toward the side with the plant. The experiment was run for 24 hours and the number of eggs on the sides with/without plant were recorded. The experiment was replicated ten times. A similar experiment was repeated with moths which had been pre-conditioned to cowpea odour by enclosing them in a cage containing the host plant, for 24 hours. The data were tranformed into logarithms of square-root and analysed.

6.2.4 Effect of host and non-host plant extracts

Host plant- cowpea, non-host plant- cotton, and wild-host plant-sunhemp were cut into very small pieces (about $3mm^2$ pieces), and 55gm of each were extracted with 400ml of methanol, chloroform and petroleum ether successively. The organic extracts obtained were concentrated to about 10ml using a rotaevaporator. Each of the extract was applied on the oviposition substrate (Crepe paper) for biological assays. The paper was demarcated into four

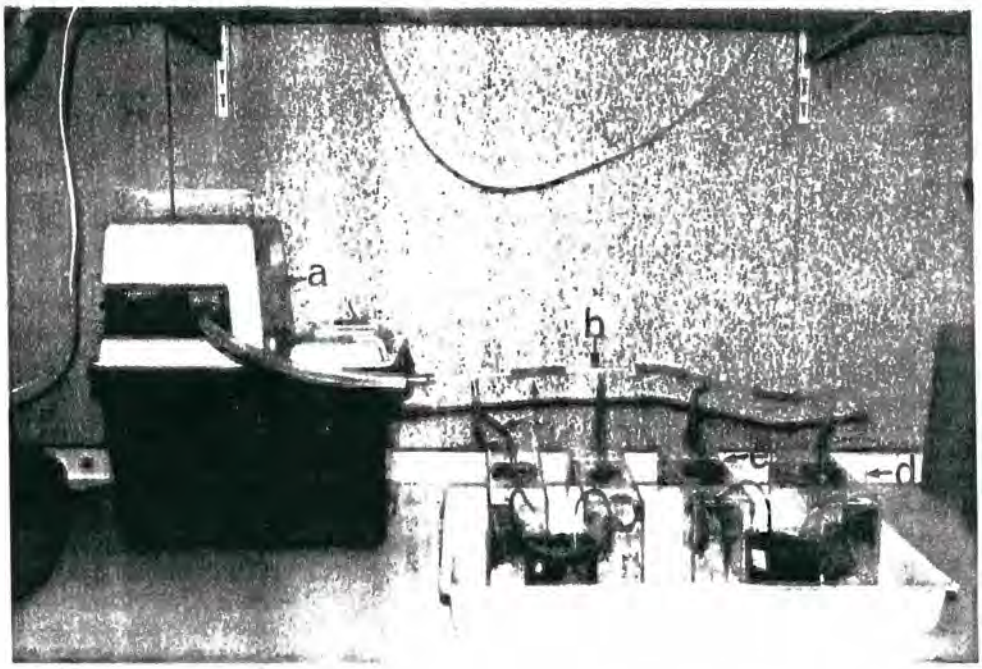


Plate 15: The Aspirator/pump used to remove air from within the cages to build an odour gradient within the cages (a=Yamato handy aspirator, b=t-joint glass tubes, c=connecting tubings, d=perforated slide sheets, and e= plastercine for securing tubings to cage outlet).

equal sections (bands) and the extract from each plant was applied onto three of the sections. The solvent minus the extract was applied to the fourth section as a control. For example, in the case of the chloroform extracts from the three plants, each of the three bands was treated with the chloroform extract from a different plant (CC1-cowpea extract, CC2- cotton extract, CC3- sunhemp extract) and the control band was treated with the chloroform solvent (Plate 16). This experiment was carried out in Nairobi and Mbita. At Mbita the temperatures and humidity were not controlled as in Nairobi. There were eight replicates in Nairobi and ten in Mbita per experiment. The data (egg counts) were transformed into logarithms of square-root and subjected to analysis of variance and the means per plant, per solvent, and per location compared by LSD test.

6.3 RESULTS

6.3.1 Effect of moisture

The moths' oviposition responses on the moist filter paper are presented on Table 9. There was no significant difference between the numbers of eggs laid on the moist paper and those laid on the dry paper at P=0.05.

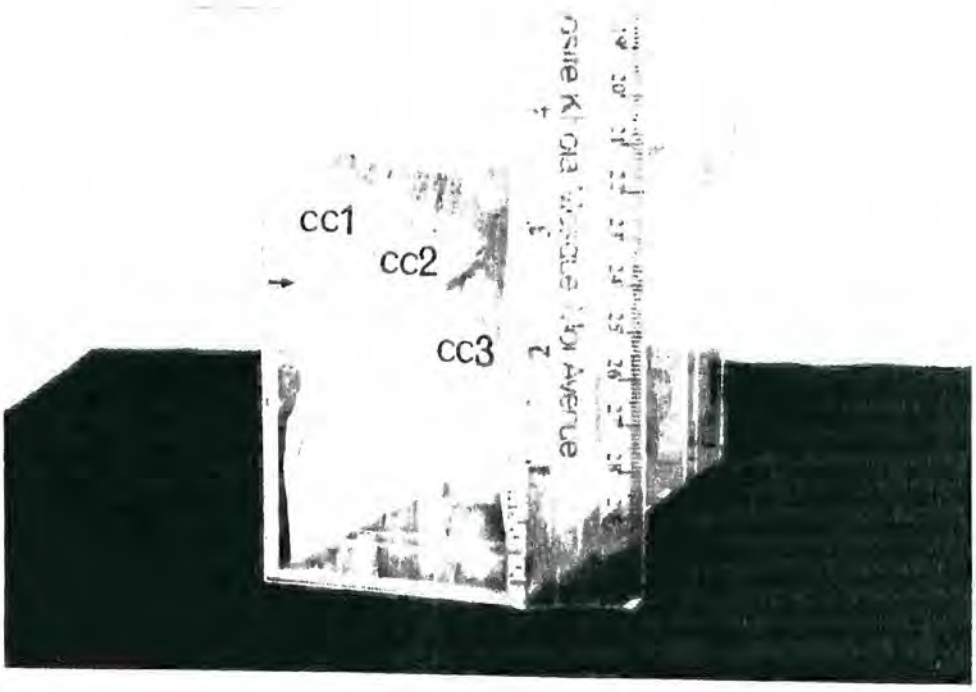


Plate 16: Set-up for plant extract test using crepe paper (arrow); cc1, cc2, cc3=sections treated with cowpea, cotton and sunhemp extracts respectively, cc4 covered partly by ruler.

Table 9:

Maruca testulalis response to moisture

N	Variable	Mean No of eggs/paper ± S.E.	*T	Prob>|T|
30	Wet paper	3.07 ± 1.08	0.22	0.82
30	Dry paper	3.83 ± 1.50		

*Students t-value for comparison of variables.

Table 10:

Oviposition response of M. testulalis to texture (rough/smooth surfaces)

Sides	+Mean No. of eggs/side ± S.E (n=6)
*1	8.37 ± 5.06a
2	5.40 ± 2.95ab
3	2.43 ± 0.99ab
4	3.13 ± 1.52ab
5	0.83 ± 0.48b
6	1.33 ± 0.66b

*Side with a rough surface
+Means with same letters are not significantly different at P=0.05 (ANOVA/SNK). Means were standardized per unit area of the surfaces.

6.3.2 Effect of oviposition substrate surface texture

Table 10 shows the mean number of eggs deposited on the surfaces that were available to the moths. Side 1, which was the rough surface, had significantly (P=0.05) more eggs deposited on it than the other five sides. Sides 2 and 4 had the next highest numbers of eggs though they were not significantly different from side 3.

6.3.3 Effect of plant odour

More eggs were laid on the side of the cage bearing the cowpea (host plant) than those sides where cotton or sunhemp was placed (Table 11). A significant difference in egg numbers was recorded between the test and control in cotton, but in cowpea and sunhemp there were no significant difference (Table 11). Comparing the means of the three plants, cowpea versus cotton and cowpea versus sunhemp showed significant differences (Table 12). The difference was not significant when cotton and sunhemp were compared. In the second set of experiments, where moths have been conditioned to cowpea odour, the moths' response to cowpea was significantly higher (P=0.05) , but unconditioned insects showed no obvious preference for the cowpea (Table 13).

Table 11:

Influence of host and non-host plant odour on the oviposition of M.testulalis

Plant	Mean egg numbers deposited per plant(x±S.E) n=30		*T	Prob|T|
	Test	Control		
Cowpea (Host plant)	30.63 ±9.35	22.67 ± 7.50	0.79	0.4382
Cotton (Non-host)	3.07 ± 1.24	18.40 ± 6.99	5.34	0.0001
Crotalaria (Wild-host)	6.83 ± 2.18	10.23 ± 3.93	0.09	0.9269

*Student t value

Table 12:

Comparing the mean eggs laid on the host, non-host and wild host plants

Plants	Mean difference (x ± S.E)	*T	Prob|T|
Cowpea/Cotton	0.62 ± 0.16	3.88	0.0006
Cowpea/*Crotalaria*	0.39 ± 0.16	2.43	0.0216
Cotton/*Crotalaria*	0.23 ± 0.14	1.59	0.1217

*Student t value

Table 13:

Influence of host plant odour on M.testulalis oviposition using an aspirator/pump

Insects	Mean number of eggs deposited/side (x±S.E)		*T	Prob>|T|
	Side 1 (Plant odour)	Side 2 (No odour)		
Unconditioned moths	0.57 ± 0.43	1.86 ± 0.88	1.61	0.1580
Conditioned moths	12.60 ± 3.58	5.20 ± 2.51	2.85	0.0190

*Student t value

6.3.4 Effect of host and non host plant extracts

Although there was no significant difference between the control and the test plants in Nairobi, extracts of the wild host-sunhemp, attracted the highest number of eggs in both locations (Table 14). However, at Mbita, the difference in egg numbers influenced by the plants extracts were significantly (P=0.05) different from the control in methanol and chloroform extracts; petroleum ether showed no difference amongst the test plants.

There was significant difference between the solvents used in the extraction at the two locations. (Table 14). Plant extracts of chloroform stimulated more oviposition in the moths in Nairobi, while petroleum ether had a higher number of eggs at Mbita location.

6.4 DISCUSSION

The fact that *M. testulalis* females deposited more eggs on the dry surface, although not significantly different, than on the moist surface, suggests that moisture has no positive influence on oviposition of the moth though it has to be borne in mind that in this experiment there was no way of controlling the degree of moisture on the filter paper. However, in other pyralidae, moisture appears to play a significant role in

Table 14:

Effect of plant extracts on the oviposition of M. testulalis in two locations

a) *Nairobi location (n=5)

Test plants	Egg numbers per solvents ± S.E.		
	Methanol	Chloroform	Petroleum ether
cowpea	8.8 ± 2.8	14.6 ± 2.5	16.0 ± 4.6
cotton	6.8 ± 1.7	11.6 ± 1.6	6.8 ± 2.1
sunhemp	21.8 ± 13.5	27.8 ± 8.3	13.6 ± 5.9
control	6.0 ± 2.3	37.0 ± 21.6	12.0 ± 5.6

b) Mbita location (n=10)

cowpea	3.5 ± 1.5b	6.7 ± 2.5a	9.0 ± 4.3
cotton	3.9 ± 1.4b	2.8 ± 1.8b	6.1 ± 1.9
sunhemp	7.0 ± 3.6b	3.7 ± 1.4ab	12.0 ± 4.8
control	17.1 ± 5.6a	7.4 ± 2.7a	11.0 ± 5.6

Means in the same column having same letters are not significantly different

*No significant difference was observed in Nairobi values.

oviposition behaviour. Hattori and Sato (1983) suggested that moisture of the oviposition substrate act strongly on the oviposition response of *E. zinckenella*. Water vapour was implicated as causing an alighting response on the substrate by ovipositing females and provoking abdomen-bending walk or ovipositor tip scanning of the substrate surface (Hattori, 1988).

The laying of more eggs on the netting material is an indication that *M. testulalis* prefers rough surfaces as oviposition sites. On the natural host plant, eggs were placed mostly along the veins (Plate 9) which further suggests the preference for rough areas. Surface texture, especially of the host plant has been known to influence the oviposition response of many lepidopterous insects. Some preferred rough surfaces -pubescent, dimpled or grooved surfaces- (Gupta and Thorsteinson, 1960; Sparks, 1973; Fenemore, 1978; Hattori and Sato, 1983; and Sosa, 1988). The mechanoreceptors found on the ovipositor tip of *M. testulalis* may play the role of assessing the roughness or smoothness when prospecting for oviposition surfaces. Ovipositing moths were noticed to prefer laying eggs on the lower surface of the host leaf than on the smoother upper surface. Eggs laid on the lower leaf surface would avoid being washed off by rain or dried out by the scorching heat of the tropical sun and the roughness may also afford the egg firm anchorage.

The observation that more eggs were laid towards the side where the preferred host leaves were present suggests that host plant odours do influence oviposition responses in *M. testulalis*. Similar observations were recorded for the cabbage looper, *Trichoplusia ni* (Shorey, 1964), *Manduca sexta* (Yamamoto *et al.*,1969; Sparks, 1973), and *E. zinckenella* (Hattori and Sato,1983), among others. Comparison of the host and non-host plant odours strongly suggested the influence of host plant odour in stimulating oviposition in this moth, *M. testulalis*. Coupled with earlier observation made in Chapter 4, one could perceive that while the non-host, cotton, deterred the oviposition response, the host plant elicited oviposition in this moth.

The difference in results recorded in Nairobi and Mbita in the oviposition response of *M. testulalis* moths to plant extracts, was probably due to the difference in the experimental location in terms of general environmental conditions and possibly the quality of the insects. In Mbita, where the natural environmental conditions were prevalent, there was observable difference between oviposition responses to the extracts of the test plants and the control; especially with respect to methanol and chloroform extracts. *M. testulalis* females do not usually lay eggs in the presence of cotton, while

sunhemp is only an alternative host when host plant cowpea is absent. Oviposition is induced normally when cowpea is present and from earlier Chapters, direct observations showed that cotton was repellent to the ovipositing moth.

The results with extracts from host and non-host plants suggest that each of the solvents used for extraction seem to extract different compounds. Methanol extracts showed that there was significant difference between the three test plants (host cowpea, non-host cotton and wild host sunhemp) and the control; which means there was indeed some chemical which affected the ovipositing moths, although there was no significant difference amongst the test plant extracts. However more egg numbers were recorded in the wild host plant. The chloroform extracts seem to give even more interesting results with significant difference obtained between the test plants extracts. Cowpea extract had significantly higher egg numbers than the cotton and sunhemp extracts, cotton giving the lowest number. Possibly, chloroform extracts of cowpea may contain some oviposition stimulants. Petroleum ether extracted fractions stimulated higher egg numbers, but there was no significant difference between the test plants and the control. It could be possible that some factor which stimulates oviposition in *M. testulalis* may also be present in the petroleum ether fraction of the test

plants.

These results should stimulate more investigations into host and non-host plant extracts influencing oviposition in *M. testulalis*. Substances with stimulating, restraining or deterrent effect on oviposition activities may have a role in plant protection, in reducing pests below the "economic injury level". The active chemical substances in the chloroform and petroleum ether fractions could be isolated and identified; and more behavioural bioassays undertaken to comfirm their effect on the oviposition behaviour of *M. testulalis*.

It is noteworthy to mention that many insects depend on some chemical or physical factors to perform various life activities including oviposition. For some insect pests, chemicals that can control their behaviour either directly or indirectly are available. These chemicals are basically present in the plants and are therefore environmentally friendly. However a lot of background information on the behaviour of a pest is essential to enable the formulation of a potential non-toxic behaviour controlling chemical which can be integrated into other control measures. Results obtained in these studies could contribute towards achieving this goal.

CHAPTER 7

OVIPOSITION RESPONSES OF M.TESTULALIS ON ARTIFICIAL SUBSTRATES

7.1 INTRODUCTION

There is the need for a satisfactory non-plant oviposition substrate which could increase the efficiency of *M.testulalis* mass production. Currently, potted cowpea plants are still being used as the main oviposition substrate for the collection of eggs. The use of cowpea plants for oviposition purposes require a good deal of labour to maintain. Such plants do not survive very well in the oviposition cages and must be replaced fairly often with new plants in order to maintain freshness. The possibility of using an artificial oviposition surface for the mass rearing of *M. testulalis* had previously been investigated by Ochieng and Bungu (1983). In that work, the filter paper was found to be preferred amongst the other substrates tested. The other substrates tested included hand towel, wax paper, parafilm and polythene sheet. The purpose of this exercise was to identify some material that can be developed into an efficient artificial oviposition substrate. Experiments were

therefore conducted to test the oviposition response of gravid *M. testulalis* moths on:

(i) Filter paper, Whatman No.91

(ii) Other substrates: aluminuim foil, butter (wax paper) and crepe Papers

(iii) Potential artificial substrates compared with host plant (cowpea).

7.2 MATERIALS AND METHODS

7.2.1 Filter paper

The inner surface (about half) of a white plastic cylindrical container measuring 13 x 13 cm (diameter x height) was lined with moist filter paper. The base of the filter paper was immersed in distilled water covering the bottom floor of the plastic container. The filter paper was to serve as oviposition surface. The top open end of the container was covered with mosquito netting material to prevent moths from escaping but allowed adequate ventilation. The oviposition chambers so made are shown in Plate 17. A pair of moths (1F:1M) were released in each chamber on their first day of emergence. They were provided with 10% sucrose solution which was applied to cotton wool placed in petri dishes from which the moths imbibed the sucrose solution. The moths were transferred daily at 1000 hours (lights-on) into a fresh

Plate 17: Cylindrical containers (oviposition chambers)

oviposition chamber. The eggs deposited by the females on the surfaces of the oviposition chamber were counted daily until the females died. This experiment was replicated six times. The egg counts were expressed per unit area of the surfaces.

7.2.2 Aluminium foil, crepe and butter papers

Choice and non-choice experiments were carried out using 10 pairs of one-day old moths. In the choice experiment, the moths had three alternatives for oviposition. The three sides of the cage were lined with the test materials: aluminium foil, crepe and butter papers measuring 15 x 18 cm (Plate 18). In the no-choice situation all the three sides of the cage were lined with one of the three test materials. The test materials (oviposition substrates) were replaced every two days. They were examined for eggs with the aid of a Wild M5 stereo-microscope and the eggs observed were counted and recorded. The oviposition responses were observed for 16 days, each experiment with three replicates and repeated twice. The eggs collected from each substrate were expressed as percentages of the total number of eggs deposited.

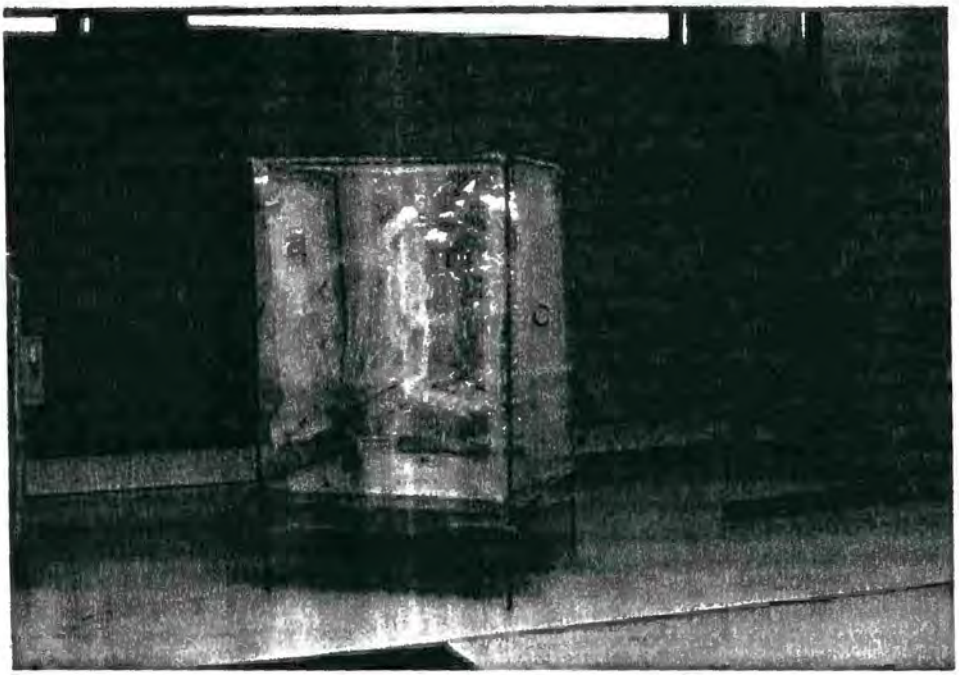


Plate 18: Cage used for testing oviposition response on Crepe paper (a), Aluminium foil (b) and Butter paper(c)

7.2.3 Artificial substrates compared with host plant

The test materials promising as potential oviposition substrates (crepe and butter papers), were presented to moths together with the host plant, cowpea. Specimens of the papers with dimension of 15 x 18 cm were stuck onto the opposite sides of 23 x 23 x 36 cm cage using transparent adhesive tape, while the leaves of a two-week old potted host plant (cowpea) was introduced through a slit at the back of the cage. Any opening left at the point of entry of the plant was sealed with adhesive tape to prevent moths from escaping. Seven pairs of one-day old moths (7M:7F) and 10% sucrose solution applied on cotton wool were introduced into the cage. Moths were expected to mate by the second day after emergence and commence oviposition on the third day; so the papers and host plant were changed on the fourth day and every other day. The numbers of eggs laid per paper/plant were recorded. The experiment ran for 14 days by which time egg-laying was expected to diminish. The experiment was replicated eight times. The data collected were compared using analysis of variance (ANOVA) with the significance of differences determined by Student-Newman Keuls (SNK) test. Prior to analysis, the data were transformed into log(square root) scale.

7.3 RESULTS

7.3.1 Filter paper

The egg-laying pattern observed on the filter paper was similar to that reported in an earlier chapter (4.3.3). Eggs were laid singly and also in clusters of two to eight, scattered in the oviposition chamber and on the filter paper. Egg-laying started two days after mating took place. About 77% of the eggs were deposited on the other surfaces (the cage, net cover and sucrose dish). Only about 23% of the total number of eggs laid were deposited on the filter paper (Table 15). Mean number of eggs laid per day per female was 28 ± 2. The pre-oviposition period was between 4 and 17 days (mean = 9 ± 2 days), while the oviposition period was 5 to 17 days (mean = 10.5 ± 2 days). The moths lived for 14-35 days (mean = 25.3 ± 3 days) (Table 16).

7.3.2 Aluminium foil, crepe and butter papers

M. testulalis females deposited eggs on all the three test materials, both in choice and non-choice situations. However more eggs were deposited on crepe paper than on any of the other two papers and the egg developed in a normal manner (Plate 19). In all cases, more than 40% of the total number of eggs laid, were deposited on the crepe

Table 15:

Number of eggs deposited on filter paper

	No. of eggs deposited per unit area of surfaces ($10^{-2}cm^2$)		
Replicate	Filter paper	*Others	Total
1	4.5	31.5	36.0
2	9.9	54.4	64.3
3	7.4	79.5	86.9
4	12.8	80.2	93.0
5	23.0	37.7	60.7
6	31.1	13.5	44.6
Total	88.7	296.8	385.5
Mean	14.8 ± 4.2	49.5 ± 11.0	
%Laid	23	77	

*Include chamber, net and Petri dish surfaces

Table 16:

Mean number of eggs laid per female and oviposition periods

Replicate	Mean egg nos./day	Longevity (days)	Pre-Ov. (days)	Ov. (days)
1	29.8 ± 12.1	21	6	5
2	32.7 ± 13.6	14	4	8
3	26.1 ± 7.1	31	5	14
4	22.5 ± 6.7	35	17	17
5	35.5 ± 8.7	29	11	13
6	22.3 ± 5.7	22	11	6
Mean	28.2 ± 2	25.3 ± 3	9 ± 2	10.5 ± 2

Pre-Ov = Pre-oviposition period; Ov = Oviposition period

Plate 19: M. testulalis eggs developing into black heads on Crepe Paper

paper followed by butter paper (Table 17).

7.3.3 Artificial substrates compared with host plant

In these tests more eggs were deposited on the host-plant than on the two test papers presented to gravid *M. testulalis* moths. The mean number of eggs deposited on the host plant was significantly higher than on the papers, except on Days 3 and 5 when crepe paper compared favourably with the host plant than butter paper did (Table 18). The overall response of the moths to the three substrates is presented on Fig.9. There was significant differences between the three substrates. About seventy-seven percent (76.5%) of the total number of eggs laid was deposited on the host plant while crepe paper attracted 18.5% and butter paper attracted the least with just 5%.

Table 17:

Total eggs laid on the various test materials

OV SBT	No-Choice Situation			Choice Situation		
	TT	MN ± S.E.	TT%	TT	MN ± S.E.	TT%
Aluminium Foil	352	117±9	25.40	145	36±5	25.22
Crepe Paper	691	230±40	49.86	262	66±10	45.57
Butter Paper	343	114±16	24.75	168	42±13	29.22

OV SBT =oviposition substrate; TT =Total; MN =Mean

Table 18:

Mean number of Eggs deposited on the host plant and test papers (crepe and butter papers) per ovipositing day

Oviposition Days	Oviposition Substrate ± S.E.*		
	Cowpea	Crepe Paper	Butter Paper
1	137 ± 46a	17 ± 6b	7 ± 3b.
2	795 ± 167a	90 ± 21b	21 ± 6c
3	114 ± 28a	48 ± 11a	3 ± 1b
4	220 ± 29a	77 ± 11b	10 ± 3c
5	159 ± 24a	110 ± 29a	13 ± 4b
6	242 ± 44a	61 ± 12b	57 ± 11b

*Means in the same row with same letters are not significantly different.

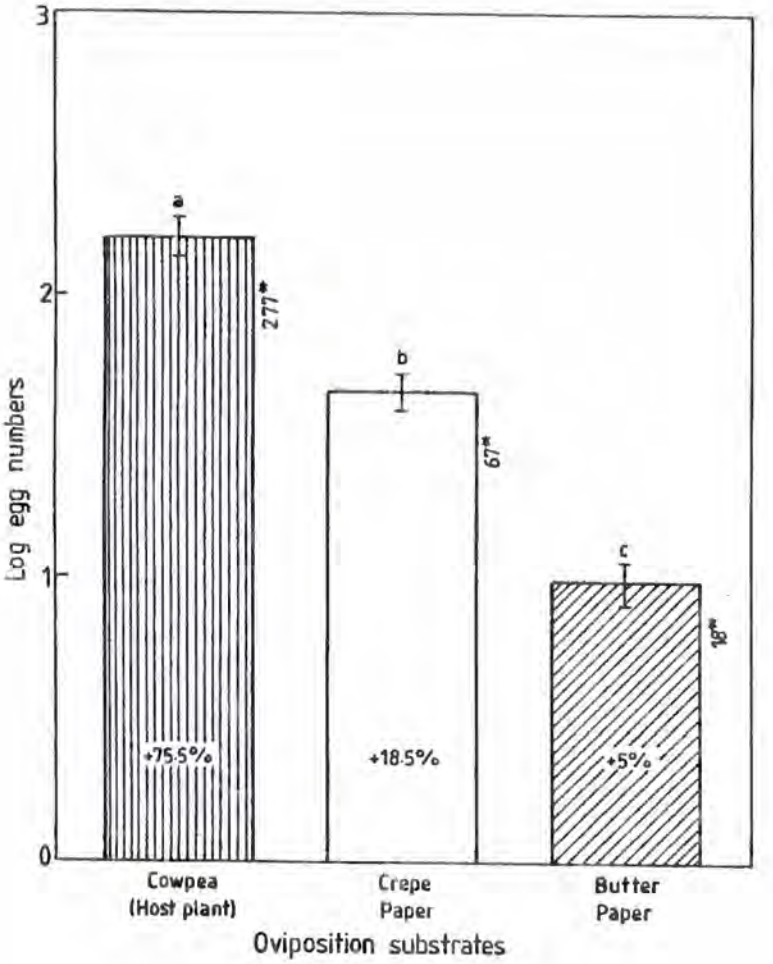


Fig. 9. M. testulalis response to the papers (Crepe and Butter papers) compared with Host plant (Cowpea). Bars with different letters are significantly different at P = 0.025 (ANOVA/SNK). *Actual mean egg numbers and +percentages are given on graph

7.4. DISCUSSION

The surface of crepe paper is rough with small/tiny grooves while the other two test materials have relatively smooth surfaces. It is possible that the rough texture of the crepe paper stimulated the gravid moths to deposit more eggs on it than on the smooth surfaces of the other two test materials. As mentioned earlier, surface texture plays a significant role in moth oviposition behaviour (Hattori and Sato, 1983). For instance, *Plutella maculipennis,* the diamondback moth laid more eggs on a pebbled, grooved or pitted polyethylene surface than on a smooth surface of the same material (Gupta and Thorsteinson, 1960).

In the presence of the host plant, crepe paper had significantly better oviposition responses than butter paper. The presence of the host plant may have enhanced the oviposition on the papers, but the higher numbers of eggs deposited on the crepe paper may have been influenced by its surface texture. Crepe paper could be suggested for use in collecting *M. testulalis* eggs in the mass rearing of the moth. This is probably the first time this paper has been tested for *M. testulalis* oviposition. Crepe paper could be a satisfactory substitute for host plant leaves in the mass rearing of *M. testulalis*. It is

suggested that aqueous extracts from the host plant could be applied on suitable artificial substrates to enhance increased egg deposition by the moths.

In recommending crepe paper as a non plant potential oviposition substrate for the collecting of *M. testulalis* eggs for mass rearing, further tests to properly assess the hatchability of eggs on the paper may be required. From observations during these experiments, eggs were seen to hatch by the third day after deposition.

CHAPTER 8

SUMMARY

1. The oviposition behaviour of *M. testulalis* on host (cowpea), non-host (cotton), wild host (sunhemp) plants and artificial oviposition substrates (filter, butter, crepe and aluminium foil papers) was studied

2. *M. testulalis* deposited fertile eggs on all three plants in choice tests. The distribution of eggs were 40%, 19% and 12% for cowpea, cotton and sunhemp respectively.

3. Crepe paper evoked more egg numbers than all the papers tested in choice and non-choice experiments. It compared favourably with the host plant when examined in choice test together with butter paper, attracting 19% of the total egg numbers as compared to 5% on butter paper.

4. Eggs of *M. testulalis* were laid singly as well as in batches of two to sixteen. Peak oviposition was on the seventh to eighth day.

5. Oviposition activities commenced two hours after lights-off; peak oviposition occurring at 2200-2400 hours i.e. six hours after lights-off at which time 60% of the total number of eggs were laid. No more eggs were deposited after 0600 hours.

6. During observation of the oviposition process, gravid moths exhibited several behavioural events:-antennae waving, flight, walking, sitting or pausing, abdomen bending walk, ovipositing and occasional feeding which occurred in various sequences.

7. Three bouts of oviposition were recorded in a six-hour observation period, each bout lasting 6-18 minutes, with extended periods of rest inbetween (32-156 minutes). More eggs were laid at the second bout.

8. The antennae and the ovipositor tip were the main organs involved in the choice of oviposition site. The tarsi probably plays some role because of the amount of walking during the oviposition process.

9. Ovipositor tip came into play at the final stage of egg deposition when this organ was used extensively to scan the immediate oviposition surface prior to depositing an egg.

10. In field observations, *M. testulalis* gravid moths started visible movements at 1920 hours as darkness set in; mainly antennae waving accompanied by flight activities.

11. Oviposition activities in the field commenced at 2140 hours each day. Flight always followed abdomen bending and eggs were deposited in 60% of such manoeuvres.

12. In combinations of host and non-host plants in field cages, cotton/cowpea combination discouraged egg-laying by gravid moths. More eggs were recorded in the cowpea/cowpea combination.

13. The spatial distribution pattern of eggs on cages and leaves was found to be contagious. The dispersion index (I_D) values obtained were significant at P=0.01 hence distribution of eggs was not random but seem to aggregate.

14. The value of the index of aggregation (K) was less than one which indicated a high degree of aggregation of eggs.

15. Light and scanning electron microscopy studies were carried out to examine the female antennae and ovipositor tip.

16. The antennae of *M. testulalis* moths were 11.5 mm long consisting of 77 and 78 antennal segments in males and females respectively.

17. The proximal segments are shorter (80-140um) and wider (120-152um) than the distal segments which are longer (96-160um) and narrow (40-56um) resulting in the filiform shape of the antennae in both sexes.

18. Electron microscopy revealed a reticulate antennal surface covered with numerous sensilla. Five main types of sensilla were found on the antennae.

19. The size, surface sculpture and manner of protrusion from socket and cuticular pits on the antennal surface differed among the sensilla types.

20. The sensilla types included sensilla trichodea (labelled a and e), coeloconica (type b), basiconica (types d and g) and sensilla styloconica (type f). Sensilla trichodea was most common and numerous.

21. Types a and e are mechanoreceptors while the others (b, d, f and g) are probably chemoreceptors suggested to be responding to odours of food and oviposition site.

22. The ovipositor of *M. testulalis* is tube-like and retractile with a flat tip consisting of two valves covered with a large number of sensilla resulting in the hairy appearance. The number of sensilla is approximately 236.

23. Two main types of sensilla were found on the ovipositor tip, designated I and II.

24. Type I sensilla are basally smooth tapering into sharp ends covered on one side with microlobes giving the sensilla a serrated or saw-like appearance. They are numerous (about 90%) and are of different lengths. They are considered to be involved in mechanoreception.

25. Type II sensilla are robust and have transverse or annular ridges on their surfaces with a blunt tip. These sensilla are contact chemoreceptors and are strategically placed on the ovipositor tip. Very few of these sensilla are present.

26. Experiments on the influence of plant physical and chemical characteristics on the oviposition of *M. testulalis* revealed that texture, odour and plant extracts stimulate oviposition in this moth.

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APPENDIX 1

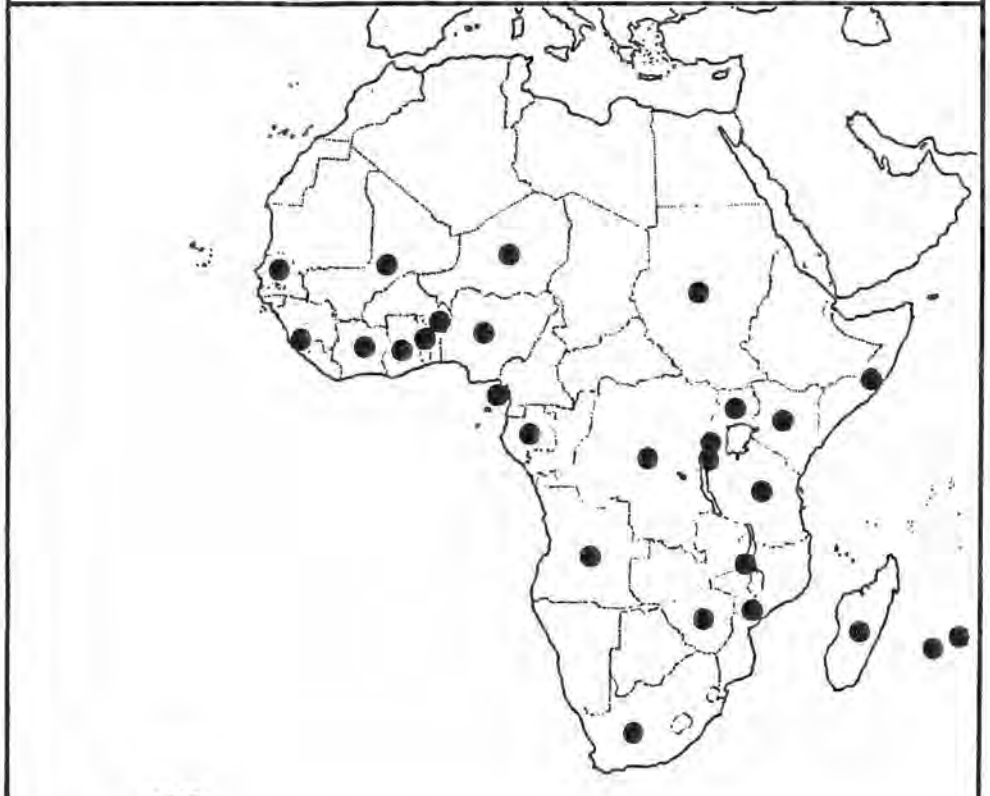
IAPSC: SCIENTIFIC SECRETARIAT

DISTRIBUTION MAPS OF MAJOR CROP PESTS AND DISEASES IN AFRICA

CPI: SECRETARIAT SCIENTIFIQUE

CARTES DE REPARTITION DES PRINCIPAUX ORGANISMES NUISIBLES DES VEGETAUX EN AFRIQUE

Map/Carte no. 240



1985

Maruca testulalis (Geyer)

Attacks Phaseolus, Cajanus, Canavalia, Dolichos, Vigna, Glycine and other legumes.

Attaque: Phaseolus, Cajanus, Canavalia, Dolichos, Vigna, Glycine et d'autres légumineuses.

See overleaf for list of countries affected
Voir au verso la liste des pays affectés

APPENDIX 1 contd

Map no. 240

Maruca testulalis (Geyer)

Lepidoptera: Pyralidae

Beanpod borer, mung moth

Attacks *Phaseolus*, *Cajanus*, *Canavalia*, *Dolichos*, *Vigna*, *Glycine* and other legumes. For world distribution see *CIE Map* no. 351.

AFRICA

Angola	[*Co-op. econ. Insect Rep.* 16 p. 267, 1964]
Benin	[Mallamaire (45 79) p. 32]
Bioko	[BMNH]
Burundi	[Buyckx (51 435) p. 598]
Gabon	[BMNH 1946 N'Djoli]
Ghana	[66 5041; 60 4618 Accra; Forsyth (54 114) p. 94 Kumasi]
Ivory Coast	[Mallamaire (loc. cit.)]
Kenya	[71 4293, 7878-9, 7982, 7986, 7988; 70 392, 3451; 69 6052; Le Pelley (48 94) p. 87]
Madagascar	[Appert (57 388) p. 195]
Malawi	[BMNH 1914 Blantyre, 1913 Mlanje]
Mali	[Mallamaire (loc. cit.)]
Mauritius	[61 4393]
Mozambique	[Del Valle y March (57 2822) p. 55]
Niger	[Mallamaire (loc. cit.)]
Nigeria	[72 806, 3906; 71 418, 2800, 6419; 70 4050, 4725; 69 5381, 6051; 68 455, 5104, 6514; 67 4581, 5044, 5051; 66 2125, 4492; 65 311, 312, 818; 61 1191]
Reunion	[72 4231; 71 7694; Plenet (50 88) p. 178]
Rwanda	[Buyckx (loc. cit.)]
Senegal	[Risbec (38 387) p. 163 Bambey, Diourbel]
Sierra Leone	[Hargreaves (26 118) p. 514]
Somalia	[Gentry (55 328) p. 158]
Sudan	[Schmutterer (57 815) p. 161]
Tanzania	[72 2196, 3104; 56 394 Northern Province]
Togo	[Mallamaire (loc. cit.)]
Uganda	[64 364; 61 1666]
Zaire	[Buyckx (loc. cit.); BMNH 1934 Kivu (Rutshuru)]
Zimbabwe	[BMNH 1901 Umtali]

APPENDIX 2

NATIONAL MUSEUMS OF KENYA

E. A. HERBARIUM
P. O. Box 45166
NAIROBI, KENYA:
Tel: 743513

Ref No. KN,ICIPE/H 50/90, KN,ICIPE/H26/92 Date: 4 June 1992

List of plants and seeds collected and brought by Mrs. Dorcas Bawo, ICIPE, P.O. Box 30772, NAIROBI.

1.	Gossypium barbadense L.	Malvaceae
2.	Crotalaria juncea L.	Papilionaceae
3.	Vigna unguiculata L.Walp.	Papilionaceae

D. Okebiro,
for BOTANIST IN CHARGE.

Appendix 3: Oviposition response on host, non-host and wild host plants

Day	No. of eggs laid per plant			
	cowpea	cotton	sunhemp	mean/day
0	0	0	0	0
2	2	1	11	4.67e
4	29	23	35	29.00d
6	57	38	11	35.33c
8	554	180	65	266.33a
10	196	114	120	143.33ab
12	68	79	72	73.00abc
14	68	62	61	63.67bc
16	47	22	20	29.67c
18	1	0	1	0.67e
Mean/plant	102±53	52±19	40±12	

Appendix 3 contd.

ANOVA

Dependent variable:egg numbers

Source	DF	SS	MS	F	Pr>F
Model	11	5.37	0.49	29.53	0.0001
Error	18	0.30	0.12		
Total	29	5.66			

R-square	C.V	Root MSE	Mean
0.95	20.20	0.13	0.64(64.57)

Source	DF	SS	MS	F	Pr>F
Day	9	5.31	0.59	35.73	0.0001
Plant	2	0.05	0.03	1.62	0.2249

Appendix 4: Pattern of eggs distribution (frequency table 1)

	Cages						
	1	4	5	6	7	8	9
No of eggs.	Frequency of units						
0	28	26	14	23	7	37	25
1	4	5	10	9	5	9	6
2	3	2	6	9	6	4	4
3	5	2	5	6	10	0	7
4	5	3	2	3	5	1	5
5	1	1	2	1	3	0	2
6	3	1	2	0	2	0	0
7	1	0	1	0	4	0	0
8	1	1	3	0	2	0	0
9	0	1	1	0	2	0	0
10	0	2	2	0	1	0	0
11	0	2	1	0	1	0	0
12	0	2	0	2	0	0	0
13	0	2	1	0	0	0	0
14	0	0	1	0	1	0	0
15	0	0	0	0	1	0	1
16	0	1	0	0	0	0	1
39	0	0	0	0	1	0	0

Appendix 5: Pattern of eggs distribution (frequency table 2)

	Cages								
	1	2	3	4	5	6	7	8	9
No of eggs.	Frequency of units								
0	49	48	25	48	41	28	48	44	30
1	2	2	10	2	2	12	3	6	11
2	0	1	8	1	3	7	0	0	4
3	0	0	3	0	1	1	0	1	2
4	0	0	3	0	2	2	0	0	1
5	0	0	1	0	1	1	0	0	0
6	0	0	0	0	1	0	0	0	0
7	0	0	1	0	0	0	0	0	2
9	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	1

Appendix 6: Pattern of eggs distribution (frequency table 3)

	Cages						
	1	2	3	4	5	6	7
No of eggs.	Frequency of units						
0	28	37	25	26	37	48	16
1	3	4	7	13	3	0	11
2	7	6	8	5	3	0	2
3	0	1	5	2	2	0	5
4	3	0	2	4	3	0	4
5	4	1	0	0	0	1	4
6	1	0	3	0	1	0	1
7	0	0	1	0	0	1	3
8	1	1	0	1	0	0	0
9	0	0	0	0	1	0	0
10	1	1	0	0	0	0	2
11	1	0	0	0	1	0	0
12	0	0	0	0	0	0	1
14	0	0	0	0	0	0	1
15	1	0	0	0	0	0	1
27	1	0	0	0	0	0	0
42	0	0	0	0	0	1	0

Appendix 7: Pattern of eggs distribution (frequency table 4)

No of eggs	Frequency of units
0	18
1	4
2	4
3	5
4	4
5	4
6	7
7	1
8	1
9	1
10	2
11	1
15	1
18	1
30	2

Appendix 8: Spatial analysis of eggs distribution (n=51)

Set	cages	x	S^2	I_D	K	/
1	1	1.63	14.82	454.60	0.201	6.261
	4	3.20	64.00	1000.00	0.168	12.291
	5	3.24	41.60	641.98	0.274	12.445
	6	1.22	5.68	232.79	0.334	4.686
	7	4.80	106.94	1113.96	0.226	18.437
	8	0.41	1.90	231.71	0.113	1.575
	9	2.35	100.57	2139.79	0.056	9.026
	Mean	2.41	53.53	1110.58	0.114	9.257
2	1	0.04	0.16	200.00	0.013	0.154
	2	0.07	0.47	335.71	0.012	0.269
	3	1.16	10.23	440.95	0.148	4.456
	4	0.08	0.47	293.75	0.016	0.308
	5	0.59	8.20	674.92	0.046	2.266
	6	0.82	5.95	362.80	0.131	3.150
	7	0.06	0.24	200.00	0.02	0.230
	8	0.18	1.12	311.11	0.034	0.691
	9	1.08	20.31	940.28	0.061	4.148
	Mean	0.45	6.05	672.22	0.036	1.728

Appendix 8 contd.

Set	cages	x	S^2	I_D	K	/
3	1	2.47	63.48	1285.02	0.10	9.487
	2	0.82	10.63	648.17	0.069	3.150
	3	1.39	9.68	348.20	0.233	5.339
	4	1.04	6.88	330.77	0.185	3.995
	5	1.04	14.66	704.81	0.079	3.995
	6	1.06	98.93	4666.51	0.011	4.071
	7	3.02	39.50	653.97	0.250	12.291
	Mean	1.55	36.57	1179.68	0.069	5.954
Leaf		4.52	40.04	487.21	0.575	17.36

Appendix 9: Number of segments on the antennae of *M.testulalis*

	Female	Male
	75	73
	76 (4)	74
	77 (3)	75 (2)
	78 (4)	77 (2)
	79 (5)	78
	80 (9)	79 (5)
	81 (2)	80 (3)
	82 (2)	-
Range	75-82	73-80
Mean	78.8 ± 0.6	77.6 ± 0.3

Appendix 10: Measurements of the antennal segments

Segment	Length (um)	Width (um)
Scape	192	288
	224	288
	216	288
	200	304
	208	296
Mean	208 ± 5.65	292.8 ± 3.19
Pedicel	96	184
	128	184
	112	168
	120	176
	152	184
Mean	121.6 ± 9.24	179.2 ± 3.19
Terminal	96	40
	88	48
	96	40
	104	40
	96	40
	96	40
Mean	96 ± 2.07	41.33 ± 1.33

Appendix 10 contd.

Proximal	96	152
	96	144
	88	144
	104	144
	120	152
Mean	100.8 ± 5.42	147.2 ± 1.96

Appendix 11: Antennal lengths of *M.testulalis*

	Male antennae (mm)	Female antennae (mm)
	12.20	11.50
	12.00	11.00
	11.00	12.50
	11.25	11.00
	11.00	11.50
	11.25	10.50
	11.25	11.00
	11.25	11.50
	10.75	11.00
	12.50	13.00
Mean	11.45 ± 0.18	11.45 ± 0.24

Appendix 12 : Sensilla lengths on the ovipositor tip of *M. testulalis*

Type I (um)	Type II (um)
134.78	69.23
56.52	78.85
43.48	57.69
126.09	57.69
113.04	65.38
204.35	-
86.96	-
47.83	-
282.61	-
56.52	-
65.22	-
169.56	-
56.52	-
104.35	-
208.70	-
239.13	-
100.00	-
Range=43-283	57-79

Appendix 13: Effect of oviposition substrate texture

(Mean egg numbers per side ± S.E.)

Sides	actual egg numbers	log standardized values
1 (rough)	41.83 ± 25.31	0.79
2 (smooth)	13.50 ± 7.38	0.63
3 (")	12.17 ± 4.95	0.51
4 (")	7.83 ± 3.79	0.55
5 (")	0.83 ± 0.48	0.33
6 (")	1.33 ± 0.67	0.39

ANOVA

Dependent variable:Egg numbers

Source	DF	SS	MS	F value	Pr>F
Model	10	3.43	0.34	8.61	0.0001
Error	25	0.99	0.04		
Corrected total	35	4.42			

R-square	C.V.	Root MSE	Log egg mean
0.7749	37.3463	0.1994	0.5341

Source	DF	SS	MS	F value	Pr>F
Sides	5	0.8099	0.1619	4.07	0.0077

Appendix 14: ANOVA for the effect of odour on *M. testulalis* oviposition response (cowpea plant)

Dependent variable: egg numbers (log values)

Source	DF	SS	MS	F value	Pr>F
Model	14	84.56	6.04	2.57	0.0085
Error	45	105.94	2.35		
Corrected total	59	190.50			

R-square	C.V.	Root MSE	egg mean
0.4439	80.9394	1.5343	1.8956 (26.65)

Source	DF	SS	MS	F value	Pr>F
odour	1	1.8384	1.8384	0.78	0.3815

Appendix 15: ANOVA for effect of odour on *M. testulalis* oviposition (cotton plant)

Dependent variable:egg numbers

Source	DF	SS	MS	F value	Pr>F
Model	14	106.16	7.58	19.16	0.0001
Error	45	17.81	0.40		
Corrected total	59	123.97			

R-square	C.V.	Root MSE	egg mean
0.8563	57.9790	0.6291	1.0851 (10.73)

Source	DF	SS	MS	F-value	Pr>F
odour	1	13.61	13.61	34.39	0.0001

Appendix 16: ANOVA for effect of odour on *M. testulalis* oviposition responses (sunhemp plant)

Dependent variable: egg numbers

Source	DF	SS	MS	F value	Pr>F
Model	14	54.19	3.87	3.02	0.0025
Error	45	57.62	1.28		
Corr.tt	59	111.81			

R-square	C.V.	Root MSE	egg mean
0.4846	97.2474	1.1315	1.1636(8.53)

Source	DF	SS	MS	F value	P>F
odour	1	0.0145	0.0145	0.01	0.9157

Appendix 17: ANOVA for comparison amongst the three test plants with respect to odour

Dependent variable: egg numbers

Source	DF	SS	MS	F value	Pr>F
Model	2	7.80	3.90	8.16	0.0006
Error	87	41.60	0.4781		
Corr.tt	89	49.40			

R-square	C.V.	Root MSE	egg mean
0.1578	81.7554	0.6914	0.8457 (13.34)

Source	DF	SS	MS	F value	Pr>F
plant	2	7.987	3.8993	8.6	0.0006

SNK grouping:

Plant	Mean	N
cowpea	30.63A	30
cotton	3.07B	30
sunhemp	6.33B	30

Appendix 18: ANOVA of the effect of plant extracts on oviposition (Mbita location-methanol extract)

Dependent variable:eggs

Source	DF	SS	MS	F value	Pr>F
Model	12	35.17	2.93	3.04	0.0079
Error	27	25.99	0.96		
Corr.tt	39	61.17			

R-square	C.V.	Root MSE	egg mean
0.5749	69.10	0.9813	1.4202 (7.87)

Source	DF	SS	MS	F value	Pr>F
Plant ext.	3	11.1308	3.7102	3.85	0.0204

Appendix 19: ANOVA of the effect of plant extracts on oviposition (Mbita location-chloroform extract)

Dependent variable:egg

Source	Df	SS	MS	F value	Pr>F
Model	12	44.10	3.67	10.63	0.0001
Error	27	9.33	0.35		
Corr.tt	39	53.43			

R-square	C.V.	Root MSE	egg mean
0.8253	50.4081	0.5880	1.1665 (5.15)

Source	DF	SS	MS	F value	Pr>F
Plant ext	3	3.2968	1.0989	3.18	0.0400

Appendix 20: ANOVA of the effect of plant extract on oviposition (Mbita location-petroleum ether extract)

Dependent variable: egg

Source	DF	SS	MS	F value	Pr>F
Model	12	56.99	4.75	11.28	0.0001
Error	27	11.36			
Corr.tt	39	68.36			

R-square	C.V.	Root MSE	egg mean
0.8338	42.0921	0.6488	1.5413 (9.5)

Source	DF	SS	MS	F value	Pr>F
Plant ext	3	0.9097	0.3032	0.72	0.5485

Appendix 21: ANOVA of the effect of plant extract on ovipositon (Nairobi location-methanol extract)

Dependent variable:egg

Source	DF	SS	MS	F value	Pr>F
Model	7	0.2723	0.0389	0.61	0.7396
Error	12	0.7679	0.0640		
Corr.tt	19	1.0102			

R-square	C.V.	Root MSE	egg mean
0.2618	61.1444	0.2560	0.4137

Source	DF	SS	MS	F value	Pr>F
Plant ext	3	0.1208	0.0403	0.63	0.6101

Appendix 22: ANOVA of the effect of plant extract on oviposition (Nairobi location-chloroform extract)

Source	DF	SS	MS	F value	Pr>F
Model	7	0.1501	0.0214	0.91	0.5309
Error	12	0.2831	0.0236		
Corr.tt	19	0.4333			

R-square	C.V.	Root MSE	egg mean
0.3465	24.8668	0.1536	0.6177

Source	DF	SS	MS	F value	Pr>F
PLant ext	3	0.0825	0.0275	1.17	0.3633

Appendix 23: ANOVA of the effect of plant extracts on oviposition (Nairobi location-petroleum ether extract)

Dependent variable:egg

Source	DF	SS	MS	F value	Pr>F
Model	7	1.0386	0.1484	5.90	0.0038
Error	12	0.3017	0.0251		
Corr.tt	19	1.3404			

R-square	C.V.	Root MSE	egg mean
0.7749	36.1664	0.1586	0.4385

Source	DF	SS	MS	F value	Pr>F
Plant ext	3	0.0892	0.0297	1.18	0.3574

Appendix 24: Overall ANOVA for oviposition response on test materials (Papers/plant substrates)

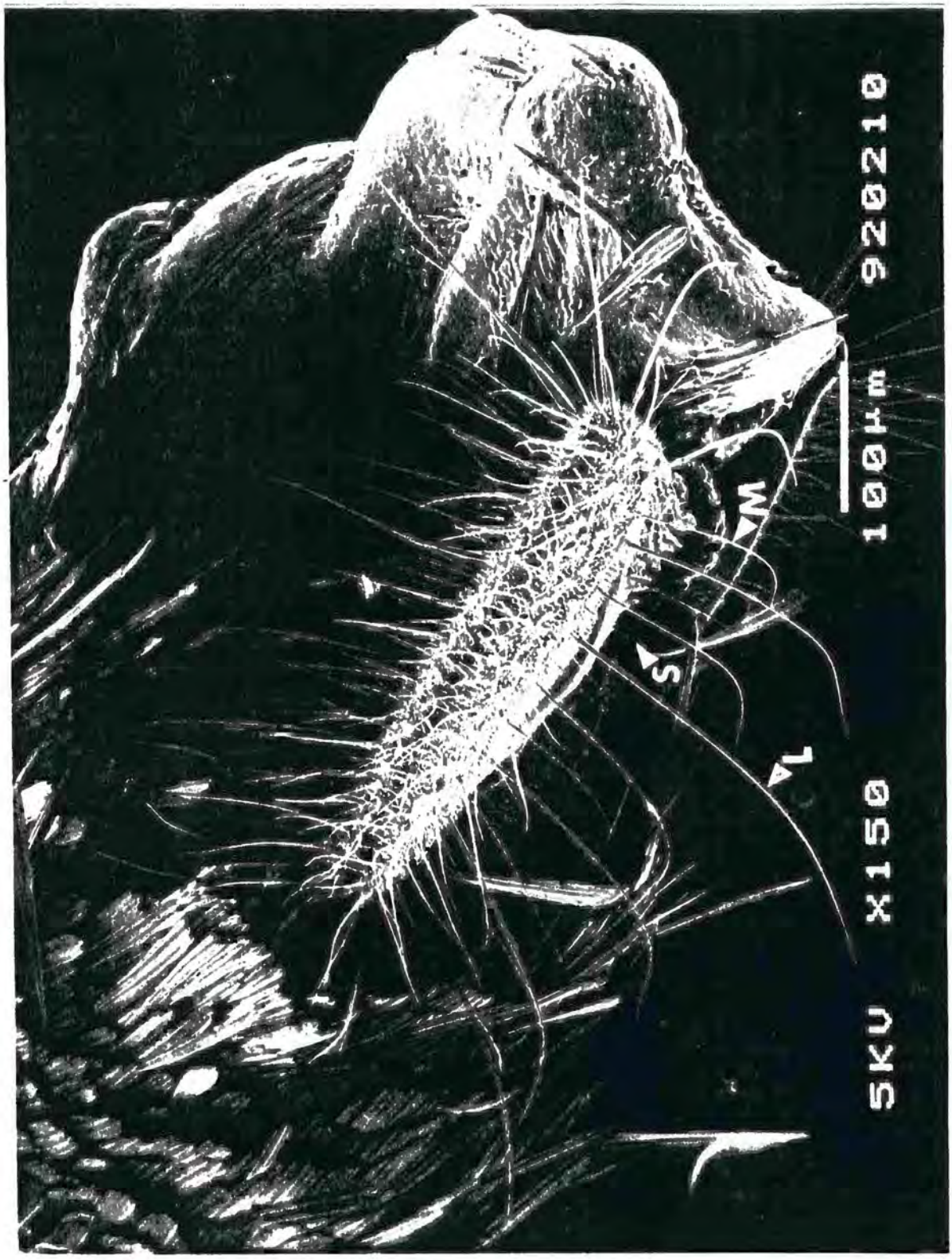
Dependent variable:egg numbers (logSqrt)

Source	DF	SS	MS	F value	Pr>F
Model	14	9.1263	0.6519	21.17	0.0001
Error	129	3.9730	0.0308		
Corr.tt	143	13.0992			

R-square	C.V.	Root MSE	egg mean
0.6967	19.8443	0.1755	0.8844

Source	DF	SS	MS	F value	Pr>F
Days	5	2.1284	0.4257	13.82	0.0001
Treatment	2	6.6900	3.3450	108.61	0.0001

Appendix 25: A complete ovipositor of *M. testulalis* from ventral aspect of abdomen (L, M, and S= Long, medium and short type I sensilla)



Appendix 26: Host plant of *Maruca testulalis* (Taylor, 1978)

Family	Plant species
Papilionaceae	*Vigna unguiculata*
"	*V. mungo*
"	*V. radiata*
"	*V. triloba*
	Cajanus cajan
"	*C. indicus*
"	*Crotalaria juncea*
"	*C. mucronata*
"	*C. incana*
"	*Arachis hypogaea*
"	*Dolichos lablab*
"	*Dolichos.* spp.
"	*Phaseolus vulgaris*
"	*P. lunatus*
"	*Psophocarpus tetragonolobus*
"	*Sphenostylis stenocarpa*
"	*Gliricidia sepium*
"	*Vicia faba*
"	*Stizolobium* spp.
"	*Mucuna* spp.
"	*Tephrosia candida*
"	*T. purpurea*

Appendix 26 contd.

Family	Plant species
Caesalpinaceae	*Poinciana*
Pedaliaceae	*Sesamum* spp.
Malvaceae	*Hibiscus* spp.
Mimosaceae	*Esclerona dolabriformis*